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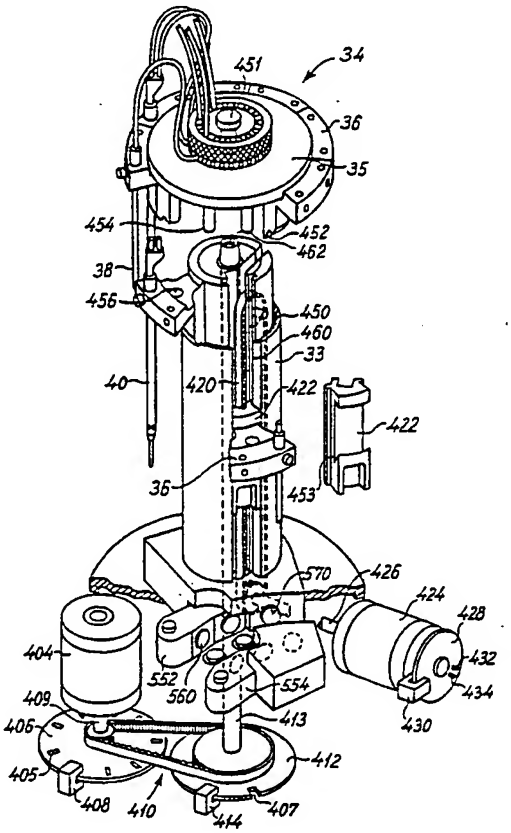
(57) Abstract

An apparatus for performing unit operations involving the handling of liquid samples comprises a number of sample containers (32), each having a portion in which a liquid sample can be contained, as well as means (38) for adding liquid to the sample containers and means (40) for removing liquid from the sample containers. When oscillatingly moving the holding means (28) holding the sample containers, some of the samples may be stirred by agitating their containers, while the samples in the remaining sample containers are not stirred to any substantial degree due to the sample containing part of these containers not being agitated to any substantial degree. The oscillating movement preferably takes place at different velocities in the two directions. High precision is obtained when dosing an amount of liquid from a pipette (38) when the pipette is given an upward acceleration at the end of a pipetting procedure so as to release from the pipette tip any part of any liquid drop in excess of an amount determined by the upward acceleration. Low carry-over due to flushing of and removal of liquid from a number of sample containers using the same suction pipette (40) is obtained using suction pipette means for sucking the liquid from the containers in combination with flushing liquid supply means (40) adapted to supply flushing liquid through an outer surface part (214) of the suction pipette means. Furthermore, a suitable pipette holding (36) and moving (422) means is disclosed for holding a plurality of pipettes and for moving any one of these to a position in which the pipette is capable of dosing liquid to a container or removing liquid from a container.



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APPARATUS AND METHOD FOR PERFORMING UNIT OPERATIONS INVOLVING LIQUID SAMPLES

The present invention relates to a novel and precise apparatus and method for performing unit operations involving handling of liquid samples, including very small liquid samples, such as samples of size of about 500 µl. The invention is particularly useful in connection with qualitative or quantitative determinations, such as determinations of components in liquid samples, involving a reaction, such as a chemical, biological, biochemical or immunological reaction which can be detected by a chemical or physical measurement, for example: an optical measurement, such as measurement of absorbance, fluorescence or luminescence (e.g. bioluminescence as in the case of a luciferin/luciferase reaction); an electrochemical measurement, such as measurement of pH or redox potential; or another form of potentiometric or electrical measurement, such as measurement of conductivity or resistance. The reaction in question may alternatively result directly or indirectly in a product whose amount is representative of that of the component to be determined and which can be detected by an optical determination; examples hereof are a fluorescein-carrying product, a coloured product, and a fluorescent product (fluorophore). The apparatus and method of the invention permit a high degree of automation with simultaneous attainment of very high accuracy.

Thus, the present invention relates to techniques where carry-over of volumes of sample, reactant or reagent is a critical factor, and where such carry-over should be reduced to a minimum, because any carry-over in any one operation results in an error which is the greater, the smaller the sample is compared with the carry-over volume.

One of the features of the present invention is that the number of objects which come into contact with the liquid samples in connection with a measurement is reduced. This is

accomplished, e.g., by performing agitation of the samples in which reactions are performed in a manner which does not entail the introduction of any agitation bodies or other agitating or stirring means into the samples, and by performing dosing of liquids from pipettes which do not contact the samples.

In the present invention, the precision in the measurement is improved because the apparatus makes use of a novel principle for agitating a number of sample containers, a novel principle for avoiding cross-contamination when removing liquid from a number of samples using the same pipette, and a novel principle for increasing the precision when metering out or dosing small amounts of liquid.

In the following, a discussion of various possible manners of performing the above-mentioned operations, including what has been suggested in the literature, is discussed.

AGITATION

Agitation or stirring of samples may in principle be carried out in two ways: using a stirring body or agitating the sample container. When using a stirring body, such as a stirring rod, carry-over may be a problem if the stirring body is not rinsed after each stirring. In the apparatus according to the invention, stirring or agitation of the samples is carried out by agitating the sample containers.

Agitation of sample containers is disclosed in EP 0.329.183 and GB 2.081.118, which describe apparatuses in which the sample containers are placed in bearings. Agitation of the sample containers is accomplished by contacting the containers with a friction member while conveying the containers along the friction member. The containers are hereby rotated around their longitudinal axis and the contents are agitated.

DOSING

Normally, precise dosing of a liquid is performed using a pipette and a precision pump.

The reproducibility of this method is limited by the variation in the size of the drop, remaining on the pipette after dosing the liquid. This drop size may vary from a lower volume, where substantially no liquid is present on the outer surface of the pipette, to a volume such that a drop which was only slightly larger would fall from the pipette.

This variation in drop size may be reduced by reducing the outer diameter of the pipette, which would reduce the outer diameter of the drop. However, the realistic outer diameter of a pipette has a lower limit.

FLUSHING; OPTICAL MEASUREMENT

A preferred type of measurement principle for determining the concentration of a component in a sample in the context of the present invention is optical measurement.

Optical measurements on liquid samples are disclosed in EP 0.329.183, EP 0,041,378, and US 4,774,055. The methods disclosed in these three references involve the transmission of light through the sample container, whereupon part of this light is absorbed by the sample; the resulting transmitted light then impinges on a light measuring means. These methods may be used when determining the light absorption (absorbance) of the sample.

However, to determine fluorescence, as is preferred in an apparatus according to the invention, of an excited sample in a sufficiently precise manner, part of the sample should be transferred to a chamber in which no extraneous light may disturb the measurement. In order to transfer the sample to the measuring chamber, a pipette or the like has to be

brought into contact with the sample to remove an aliquot or part thereof. This operation may be a source of cross-contamination unless care is taken.

A method for decreasing cross-contamination using disposable pipette tips which are used only for one sample is disclosed in German Patent No. 40 11 584.

An alternative way of reducing cross-contamination when using the same pipette for removing liquid from a number of samples is disclosed in US 4,477,055. in this case, water is sucked up into the pipette before removing an aliquot of the sample in question. By ensuring that the two liquids are separated by air, the sample aliquot does not become polluted or diluted by the water. After delivering the sample aliquot in question, the water is then expelled, thereby rinsing the inside of the suction pipette.

DISCLOSURE OF THE INVENTION

In one aspect, the present invention relates to an apparatus for performing unit operations involving the handling of liquid samples, the apparatus comprising:

a number of sample containers, each having a portion in which a liquid sample can be contained,

means for adding liquid to the sample containers, and means for removing liquid from the sample containers,

the apparatus showing at least one of the following features a)-d):

a) holding means for holding the sample containers, the holding means having container-receiving means adapted to receive each container in male-female relationship with the container-receiving means, and means for oscillatingly moving the holding means and thereby

transferring movement from the holding means to the sample container to agitate liquid in the sample container received by the container-receiving means,

b) the means for adding liquid to the sample containers or removing liquid from the sample container comprise a plurality of pipettes arranged in pipette holding and moving means adapted to hold the pipettes and to move each individual pipette to a position relative to a sample container in which the pipette is capable of exerting its function of dosing liquid to the container or remove liquid from the container

c) the dosing pipette means are movable by pipette moving means which are adapted to confer to the pipette means an upward acceleration at the end of a pipetting procedure so as to release from the pipette tip any part of any liquid drop in excess of an amount determined by the upward acceleration,

d) the means for removing liquid from the sample containers comprise suction pipette means for sucking the liquid from the containers combined with flushing liquid supply means adapted to supply flushing liquid through an outer surface part of the suction pipette means.

A preferred embodiment of the apparatus of the invention is adapted for quantitative or qualitative determination of a component in liquid samples by performing, on individual liquid samples, a reaction which can be detected by a chemical or physical measurement or which directly or indirectly results in a product which is representative of the component and which can be detected by a chemical or physical measurement. This preferred embodiment of the apparatus further comprises measuring means adapted to perform the chemical or physical detection of the reaction or the product resulting from the reaction.

In a particularly preferred embodiment of the apparatus according to the invention, the measurement in question is an optical measurement and the measuring means is an optical measuring means adapted to perform optical detection of the reaction or the product resulting from the reaction.

Each of the above features a)-d) constitutes an important approach which can be utilized per se in connection with determinations of the above types, and as will become apparent from the disclosure herein, an apparatus according to the invention is ideally suited not only to performing unit operations in conjunction with associated chemical or physical measurements, but also simply for manipulating liquid samples with a high degree of precision for the purpose of, e.g., dilution, dividing into aliquots and/or mixing. As will be explained in the following, each of the above features, and additional features as explained below, contribute to a unique exactitude in the manipulations and/or measurements involved in the operation of an apparatus according to the invention.

When a reaction is performed on the samples, this reaction may be a conventional chemical reaction generating a product which can be detected by an optical measurement, such as determination of UV-induced fluorescence, or it may be a biological or biochemical reaction, such as an enzymatic reaction on a substrate generating a fluorophore as a reaction product. A type of reaction for which the apparatus and method of the invention are particularly suited is an immunological reaction, such as employed, for example, in an assay of the ELISA type (Enzyme Linked Immuno Sorbent Assay) for detection or determination of an antigen, antibody or hapten. The ELISA method is based on the use of antibodies against the molecule or species to be determined or detected, and the antigen, hapten or antibody is detected by means of an enzyme which is covalently coupled (also denoted linked or conjugated) either (when an antigen or hapten is to be determined) to an antibody which is specific for the antigen

or hapten in question, or (when an antibody is to be determined) to an antibody which is specific for the antibody in question.

An ELISA determination, e.g. an ELISA determination suited for performance using an apparatus according to the present invention, may, e.g., be performed as follows:

A) The antigen, hapten (the latter generally in the form of a conjugate with, e.g., a protein) or antibody to be detected/-determined is bound or immobilized by allowing it to bind immunochemically to a so-called "catching" antibody (in the case of antigen or hapten determination) or an antigen (in the case of antibody determination) which is attached covalently or non-covalently to the surface of an appropriate material. This reaction may be faster when the sample container is agitated or if the temperature is raised. The latter attached antibody or antigen may be attached, for example, to the material of the inner surface of the sample container employed, or to insoluble, magnetic particles which are capable of being immobilized within the sample container by applying a magnetic field to the outside of the container.

B) Components which are not bound to the attached antibodies or antigens are washed away. When magnetic particles are used as the attachment material, they are immobilized during the washing procedure by applying a magnetic field as described above.

C) Appropriate enzyme-linked antibodies (which may be specific antibodies, e.g. monoclonal antibodies) are then added to the sample and allowed to bind to the immobilized species (antigen, antibody or hapten) which is to be detected/determined. This reaction may be faster when the sample container is agitated or if the temperature is raised.

D) Unbound components are washed away as described in B).

E) The amount of bound specific antibody, and thus the amount of immobilized species, is then determined by adding a substance which is a substrate for the linked enzyme and which, upon enzymatic decomposition, yields a product or products which may be detected or determined (directly or indirectly) by an appropriate chemical or physical measurement, such as an optical measurement, e.g. a fluorescence measurement, or a measurement of one of the other types mentioned above.

F) A reagent that stops the enzymatic reaction in step E) is suitably added after an appropriate period of time.

G) The measurement mentioned in step E) is performed.

H) Concerning step E), an alternative way of producing a product detectable by an optical measurement is to add an additional substrate to the reaction mixture formed in step E). The product of this new reaction may then be detected by an optical measurement.

As mentioned above, "catching" antibodies or antigens (as appropriate) may, instead of being attached to magnetic particles, be attached, bound or otherwise immobilized to the inner surface of the sample containers. However, when using magnetic particles, the accessible surface area of attached antibody or antigen will typically be of the order of 10 times that obtained when using sample containers having the antibody or antigen attached to the inner surface thereof. In addition, if no agitation of the sample is performed at all, completion of the reaction of an antigen which is to be determined with the attached "catching" antibodies, or of the enzyme-linked antibody with the bound antigen which is to be determined, may take of the order of 20 hours, whereas completion of the corresponding reaction using magnetic particles may be achieved within about 15 minutes (Millán et al., Clin. Chem. 31 (1985) pp. 54-59). This effect is further amplified when agitation of the sample during the reaction is performed. Thus, the use of magnetic particles in a procedure

of, e.g., the ELISA type, such as a procedure to which the apparatus and method of the invention can contribute to the obtainment of a large sample throughput.

In the above examples, operations A) through G) are performed in a sample container, most of these operations involving transfer of liquid to or from the container. In accordance with what is mentioned above, the individual samples must necessarily be quite small, such as about 500 µl, if a relatively large number of samples are to be handled efficiently and in view of the fact that the reagents employed in the various stages of the procedure are often quite expensive. This means that precise dosing, complete transfer and efficient rinsing must be ensured if the results obtained are to be accurate.

The present invention provides measures which permit the attainment of a very high accuracy in dosing, transfer, rinsing and flushing, and, at the same time, a high degree of precision with respect to the degree of completion of the reactions in question via efficient control of agitation and of sample temperature. Control of sample temperature is made possible, in particular, by an efficiently thermostatable design obtainable due to the compact construction of the sample handling and measuring module of the apparatus according to the invention. This will become apparent from the following.

Thus, in an apparatus according to the invention, it is possible to perform operations as exemplified above with a carry-over of the order of 0.01% when using the same pipette for withdrawing liquid from a number of sample containers, and a carry-over of substantially zero when adding liquids to the sample containers. Also, an aspect of the invention comprises obtaining increased precision in dosing liquids to sample container from pipettes by careful control of the size of any drop remaining at the pipette tip, thereby increasing

the accuracy in the dosing by up to several orders of magnitude.

In the following, the individual features of the apparatus according to the invention will be explained in greater detail.

According to the present invention, the agitation of the samples in sample containers is performed by oscillatingly moving the holding means in which the sample containers are received in male-female relationship in the container-receiving means. However, while the majority of the containers in an apparatus will typically be under agitation because a reaction is performed therein, one or a few of the containers are preferably not agitated (or not agitated to any substantial extent), namely the container or containers which are close to or at the place where reagents are added thereto or liquid is withdrawn therefrom. Therefore, the agitation in these selected containers may be substantially reduced or prevented by ensuring that these selected containers are in a more loose engagement with the holding means. This is obtained when the apparatus comprises means for moving at least one container axially in relation to the corresponding receiving means between a first position, in which the sample container is received in the holding means with a relatively close fit, and a second position in which the sample receiving part of the container may be moved transversely in relation to the receiving means. When positioned in the second position, the sample-containing part of the container can be held substantially stationary while the opposite part of the container may move more freely. Although the opposite part of the container can move considerably, it will be possible to dose liquid to the container or withdraw liquid from the container when the sample-containing part of the container is kept relatively static in the second position; this makes it possible to perform the dosing or withdrawal at a time where other

containers are still being kept in the position where their contents are subjected to effective agitation.

A suitable design of the container and the container-receiving means, respectively, is one where the outer surface of each container defines a shoulder between its opposite ends, and each of the receiving means of the holding means defines a support surface for engaging with the shoulder of the container, such as illustrated in the drawing.

The holding means is suitably a plate-like member with a plurality of apertures for receiving the sample containers, where each aperture has a substantially circular outline, the diameter of the aperture at the lower surface of the holding means being smaller than at the upper surface thereof. Thus, typically, each aperture is at least partly defined by a frustro-conical surface part, the shoulder of each of the containers defining a substantially complementary frustro-conical surface part being in abutting engagement with the first mentioned frustro-conical surface part in the first position of the container. It has been found that a suitable top angle of the frustro-conical surface parts is within the range of 3°-18°, preferably 6°-13°, and more preferably within 9°-11°. To ensure easy removal of the sample container from container-receiving means, the top angle of the conical surface part of the container is normally smaller, preferably 0.1°-1° smaller, than the top angle of the conical surface part of the aperture.

In a presently preferred embodiment, the top angle of the conical surface part of the apertures is approximately 10.0° and the top angle of the conical surface part of the containers is approximately 9.7°.

The holding means is suitably adapted to oscillate or reciprocate the receiving means along an arcuate path, and the holding means thus preferably comprises a substantially horizontally arranged, substantially circular disc which is

reciprocatingly movable around its central axis. The preferred way of moving the holding means is to use a stepping motor drivingly connected to the disc, the disc preferably being mounted directly on the shaft of the stepping motor, and the stepping motor is preferably controlled so as to move with constant speed.

When agitating the samples along an arcuate path, the forces acting on the liquid in the sample containers is the centrifugal force. The magnitude of this force depends on the angular velocity of the liquid, again depending on the distance from the liquid to the centre of the movement. When the holding means is moved in a reciprocating manner and if, as preferred, the velocity in one direction is larger than the velocity in the other, the liquid parts positioned at the largest distance from the centre of the movement, preferably being the centre of the substantially circular holding means, will respond strongest to the movement. As the centrifugal force depends on the square of the angular velocity, the liquid parts positioned in the largest distance from the centre of the movement, will respond strongest to the movement having the largest velocity, which means that the liquid in the sample container will perform a rotational movement when the movement if the holding means is larger in one direction than in the other.

The velocity in one direction is typically at least 2 times higher than in the other, preferably 3-4 times higher, but there is nothing to hinder the use of velocities more than e.g. 9 times higher in one direction than in the other.

When working with immunological reactions, as in ELISA, it is advantageous to immobilize antibodies on magnetic particles in a manner known per se. Therefore, a preferred embodiment of an apparatus according to the invention comprises magnet holding means adapted to hold at least one set of magnets in a position where they induce a magnetic field in the sample-holding part of at least one sample container positioned in

its second position. The magnet holding means are suitably adapted to bring, in one position, the magnets in direct contact with the sample-holding part of the sample container positioned in its second position, and arrange the magnets in another position in which they do not contact the sample container.

In this preferred embodiment the sample receiving parts of a sample container positioned in its second position may be held substantially stationary while the rest of the sample container may follow the movements of the holding means. Prevention of movement of the sample receiving parts of the sample container in its second position is preferably accomplished by contact of the magnets with the sample container and by elevation of the sample container by the elevating means.

As the other parts of the sample container are able to follow the movements of the holding means, the sample receiving parts of the sample container are not completely stationary, but are able to rotate about an axis through the sample held by the sample container. This enables the sample container to follow the movements of the holding means substantially without agitating the sample.

In this manner the magnets are able to contact the sample receiving parts of the sample container(s) to attract and immobilize any particles in the sample(s) in question having magnetic properties while other samples are agitated.

It is often practical that the magnet holding means comprise at least two sets of magnets, adapted to be brought in contact with at least two neighbouring sample containers. In this embodiment, one set of magnets can be utilized to pre-attract the magnetic particles in order to reduce the overall processing time in connection with a measurement. Each set of magnets is suitably adapted to contact the sample container from substantially opposite sides and with opposite magnetic

poles so as to generate a suitable magnetic field in the sample container.

Furthermore, as good contact is obtained between the magnets and the sample containers, firm retention of the magnetic particles in the sample containers is quickly obtained, whereby loss of magnetic particles, and thus of the immunochemically reacting species to be determined, during the determination is minimized; furthermore, this contributes to increased sample throughput of the apparatus and method of the invention.

The apparatus according to the invention preferably comprises, as means for adding liquid samples to the sample container, pipettes fed by continuous liquid strings extending from containers containing the liquids to the tip of the pipettes, the transport of liquid preferably being performed by means of peristaltic pump means.

The present invention also relates to a novel way of performing precise pipetting of liquid into a container by means of a pipette, comprising subjecting the pipette to a movement with an upward acceleration at the end of a pipetting procedure so as to release from the pipette tip any part of any liquid drop in excess of an amount determined by the upward acceleration and allowing any such released amount to be added to the liquid in the container.

In the prior art, the precision of the pipetting is normally limited by the volume interval of drops able to remain on the tip of the pipette. By introducing this movement of the tip of the pipette, this volume interval is reduced whereby the precision of the dosing is increased.

In a preferred embodiment, at the end of a pipetting procedure, the pipette is moved upward to a first position, whereupon the pipette is subjected to downward movement, which movement is reversed by means of the upward

acceleration. This jerk-movement, and results illustrating the advantages obtained are described in Example 2.

To avoid cross-contamination, the pipette is preferably kept free of contact with the liquid in the container.

The means for removing liquid from the sample containers comprises flushing pipette means combined with flushing liquid supply means adapted to supply flushing liquid to an outer surface part of the flushing pipette means. Thereby, it is possible to remove liquid from a sample container and to flush both the flushing pipette and the sample container without moving the flushing pipette. In many known systems, the pipette would typically be moved to a washing station for rinsing or flushing after each suction procedure.

The flushing pipettes are preferably designed so that the flushing liquid supply means is adapted to supply flushing liquid to the outer surface part of the flushing pipette through at least one orifice in the flushing pipette means positioned at a distance above the tip of the flushing pipette. Thereby, it is possible to very effectively flush the parts of the outer surfaces of the flushing pipettes which have been in contact with the samples. Thus, the flushing liquid supply means suitably comprises an annular cavity surrounding the flushing pipette means along a part of the length of the flushing pipette means.

When performing e.g. an ELISA-type determination, the sample container, from which liquid has been removed, is preferably flushed to additionally rinse the contents of the sample container. This flushing method preferably comprises

- immersing the pipette into the liquid,
- sucking the liquid sample into the pipette, and
- flushing the outer surface part of the pipette having been in contact with the container by means of a flushing liquid.

The flushing pipette used in an apparatus according to the invention is able to perform this flushing without being removed from the sample container. This means that the flushing pipette comprises both means for removing liquid and means for introducing flushing liquid into the sample container.

In a preferred embodiment of a flushing pipette, the flushing liquid is supplied through at least one orifice in the pipette positioned above the outer surface part of the pipette which has been immersed in the liquid. In this way the flushing pipette is efficiently flushed, and as the flushing liquid is introduced in the sample container, the parts of the sample container, previously in contact with the sample, are also flushed.

The flushing preferably comprises at least one flushing sequence where flushing liquid is supplied in an amount which is equal to or preferably larger than the amount of liquid sample which has been withdrawn so as to secure that total outer surface part of the pipette which has been immersed in liquid is contacted by the flushing liquid.

The pipettes of the apparatus according to the invention are very suitably held and moved by means of pipette holding and moving means adapted to hold a plurality of pipettes and to move any one of the pipettes to a position in which the pipette tip is capable of entering a sample container. Thus, a preferred embodiment of an apparatus showing this feature comprises a number of pipette-holding segments each of which is capable of holding at least one pipette, a segment support means comprising a guide surface on which the segments, arranged adjacent to each other, are supported and guided, means for sidewardly moving the segments, a movable member comprising a support surface adapted to support a segment, the movable member being able to occupy a first position in which its support surface replaces and constitutes part of the guide surface, and means for moving the movable member in

downward and upward directions, each segment being sidewardly movable by the segment moving means to a position where the guide surface is replaced with the support surface of the movable member, whereby the segment becomes movable with the movable member, the movable member being movable between the first position and a second position in which the tip of a pipette held by the segment is capable of entering a sample container.

The guide surface is preferably in a substantially horizontal plane, and the downward movement is preferably a substantially vertical movement. To effectively engage and hold the pipette holding segment, the movable member preferably comprises an upper guide surface part adapted to engage an upper surface part of a segment supported by the support surface part of the movable member.

A practical way of ensuring that the pipette holding segment engaging the movable member is substantially fixed in relation to the movable member, the means for supporting the segment comprises a groove having substantially vertical-parallel edges, the movable member being movable from its first position to its second position along the groove, the segment held by the movable member being fixed in sideward direction by the edges of the groove when the movable member is not in its first position.

The guide surface of the segment support means preferably constitutes part of a substantially circular surface, the remaining part of the substantially circular surface being constituted by the support surface of the movable member when the movable member is in its first position. The sideward movement of the segments is suitably obtained by means of a rotatable top part provided with means for engaging the segments supported by the support surface so that the segments are moved sidewards in a substantially circular path when the rotatable top part is rotated, the engaging means being in slidable engagement with the segments allowing a

segment which is moved downward with the movable member to become disengaged from the top part.

The invention also relates to a sample container, in particular for use in the apparatus described above, the sample container having the features described above.

The diameter of the upper substantially cylindrical surface part of the container is preferably larger than the diameter of the lower cylindrical surface part. Thus, the diameter of the lower substantially cylindrical surface part is normally in the range of 10-15 mm, preferably in the range of 11.5-13.5 mm, such as about 12.5 mm, and the diameter of the upper substantially cylindrical surface part is typically in the range of 13-19 mm; preferably in the range of 15-17 mm, such as about 16 mm. The total length of the lower cylindrical part preferably being in the order of 25-32 mm. The lower cylindrical part preferably comprises a downwardly extending substantially hemispherical bottom part.

The sample container is suitably made of polyolefin, such as polypropylene. Fig. 13 illustrates a preferred embodiment of a sample container, also giving the dimensions of the container. The container, or at least the sample-containing part thereof, is preferably not transparent to light.

As will be understood from the above explanation, the apparatus and method according to the invention provides a totally new strategy for performing determinations of the type described:

Compared to known methods, such as ELISA machine methods, where matrices of a number of wells and the same number of pipettes are used, the method permits the use of larger samples, and at the same time permits a much better agitation and thereby diffusion in the reactions performed in the containers. Because of the use of larger samples than in ELISA machines, combined with high dosing accuracies and high

diffusion reproducibilities, genuinely quantitative and, indeed, highly accurate ELISA determinations become possible. Compared to classical determinations performed in large scale equipment, the method of the invention is unique in that all reactions are performed without transfer of samples between different containers, and needing shorter time because of the very efficient agitation of the individual containers, at the same time, as a very special feature, permitting dosing and withdrawal from containers arranged in the same holding means as the containers which are subjected to agitation, and even permitting the simultaneous agitation of some containers and dosing to or withdrawal from, other containers.

Description of a preferred embodiment of an apparatus according to the invention with reference to the drawing.

Fig. 1 shows an apparatus according to the invention in its entirety,

Fig. 2 is a detailed partly broken-away view of a pipette tower,

Fig. 3 shows the flow of liquids in the apparatus,

Fig. 4 is a detailed partly broken-away view of the bottom part of the apparatus comprising elevating means, heating means, and a motor moving the holding means,

Fig. 5 shows a sample container placed in the lower position in which it is in a relatively close fit with the holding means,

Fig. 6 shows an elevated sample container which is positioned in its second position and is contacted by a set of magnets,

Fig. 7 shows an elevated sample container contacted by a set of magnets, the sample container being in one of its extreme positions during agitation of other sample containers which are not elevated,

Fig. 8 shows the forces acting on the contents of a sample container when agitated,

Fig. 9 shows a graph illustrating the position of a sample holding means versus time during agitation,

Fig. 10 shows a partly broken-away view of a dosing pipette and a flushing pipette,

Fig. 11 shows forces acting on a drop of liquid hanging from the tip of a dosing pipette,

Fig. 12 shows the movement of a dosing pipette while the pipette is subjected to the jerk movement,

Fig. 13 is a cross sectional view of a sample container suitable for use in the apparatus according to the invention.

Fig. 1 shows a preferred embodiment of an analysis apparatus according to the invention, generally designated 10, comprising a controlling/calculating module 12, a sample handling and measuring module 14 and a keyboard or other interface 16.

The controlling/calculating module 12 comprises power supply (not shown) and controlling/calculating electronics 11.

The sample handling and measuring module 14 comprises a reagent supply module 18, two pump modules 20, each comprising up to four substantially identical pumps 22, e.g. peristaltic pumps, a valve module 24 comprising three substantially identical valves 26, a turntable 27, a pipette tower 34 and an optical measuring module 42.

A cover 15 permits efficient thermostating of the turntable 27, the pipette tower 34, and the optical measuring module 42.

The turntable 27 includes a disc-shaped holding means or turntable disc 28 comprising a plurality of holes 30 constituting container-receiving means for holding a number of substantially equally spaced sample containers 32.

The pipette tower 34 comprises a rotatable top part 35 holding a plurality of pipette holding segments 36 each adapted to hold one or two pipettes. Two types of pipettes

are shown namely a dosing pipette 38 and a withdrawal and flushing pipette 40.

The top part 35 of the pipette tower 34 is rotatably mounted on a stationary part 33 of the pipette tower 34 by means of a bolt 451 received in a thread 462 (Fig. 2) positioned in a rotatable shaft 413. Rotation of the top part 35 is accomplished by activating a motor 404 having a shaft 409 which, via a toothed belt drive 410, rotates the shaft 413 and thereby the top part 35. The rotation of the top part 35 is monitored by optical detectors 408 and 414 detecting apertures 405 and an aperture 407 positioned close to the outer edges of discs 406 and 412, respectively, fixed to the shafts 409 and 413.

Each of the optical detectors 408 and 414 comprises a light emitting part (not shown) and a light detecting part (not shown) which are oppositely positioned in two oppositely positioned arms of the detector so that light emitted from the light emitting part will be detected by the light detecting part if the light is not obstructed.

The disc 412 comprises only one aperture 407 enabling the detector 408 to generate a signal when the top part 35 is in a certain position - the starting position.

The motor 404 may be activated to rotate the top part 35 controlled by the controlling/calculating electronics 11, on the basis of the mechanical advantage of the belt drive 410 and the signals obtained from the optical detectors 408 and 414. The top part 35 may be rotated to move any of the pipette holding segments 36 to a position where it engages a slide member 422.

The lower parts of the pipette holding segments 36 are supported by a frustro-conical surface part 450, formed in the stationary part 33, with which frustro conical surface part 450 complementary surface parts 452 constituting a

sector of a frustro conical surface, formed on each pipette holding segment 36, engage. The upper part of each pipette holding segment 36 has a surface part 448 constituting a sector of a frustro conical surface and engaging with a corresponding frustro conical surface part (not shown) of an element (not shown) to be fastened to the stationary part 33.

Surface parts 453 and 449, each constituting sectors of a frustro conical surface and corresponding to the conical surface parts 450 and the upper frustro conical surface part (not shown) of the stationary part 33, are formed in the slide member 422, and, in the upper position of the slide member 422, cooperate with these frustro conical surface parts of the stationary part 33 to support the pipette holding segments 36 in such a way that they are able to move along the circumference of the stationary part 33. A condition for the circumferential movement of the pipette holding segments 36 is that the slide member 422 is in an upper position. When the slide member 422 is not in the upper position, a catch (not shown) mounted on the stationary part 33 of the pipette tower 34 engages the top part 35 and prevents rotation thereof.

When a pipette holding segment 36 is positioned in engagement with the slide member 422, the slide member 422, carrying the pipette holding segment 36, may be moved along a groove 460 extending along a longitudinal axis of the stationary part 33. When a pipette holding segment 36 has been positioned in engagement with the slide member 422, the slide member 422 is moved downward to an intermediary position, and the slide member 422 will not occupy its upper position until a new pipette holding segment 36 is to be selected. When the slide member 422 is not in its upper position, edges 461 of the groove 460 prevent the pipette holding segment 36 from moving along the circumference of the stationary part 33, whereby the pipette holding segment 36 is substantially fixed in relation to the slide member 422.

To ensure that the pipette holding segments 36 follow the rotation of the top part 35, pins 454, fixed to the top part 35, engage holes 456 in the pipette holding segments 36, thereby forcing the pipette holding segments 36 to rotate with the top part 35.

The movement of the slide member 422 is performed by means of a motor 424 rotating a shaft 426 which, by means of a toothed belt drive including a toothed belt 420, transports the slide member 422, which is fixed to the toothed belt 420.

The positioning of the slide member 422 in its upper and intermediary positions is monitored and controlled by the controlling/calculating electronics 11 on the basis of signals generated by an optical detector 430 detecting apertures 432 and 434 in a disc 428 fixed to the shaft 426.

The aperture 432 is detected when the slide member 422 is in the upper position, and the aperture 434 is detected when the slide member 422 is in the intermediary position which is preferably slightly lower than the upper position. Movement of the slide member 422 and thereby the pipette holding segment 36 below the intermediary position of the slide member 422 is accomplished by means of a sine-shaped voltage generated by the controlling/calculating electronics 11. The motor 424 is preferably a stepping motor, in which case the number of periods and the frequency of the voltage transmitted to the motor 424, define the position and velocity of the slide member 422.

In the turntable 27, rotation of turntable disc 28 is accomplished by activating a motor 502 having a shaft 504 on which the holding means 28 is fixed (Fig. 4).

A disc 506, which is fixed to the shaft 504, comprises a number of apertures 512 positioned at a small distance from the edge of the disc 506, and one aperture 514 positioned in the edge of the disc. The apertures 512 are detectable by an

optical detector 508 and the aperture 514 is detectable by an optical detector 510.

The spacing of the apertures 512 corresponds to the spacing of the holes 30 in the holding means 28.

The holes 30 are preferably frustro-conical having a smaller diameter at the lower surface of the holding means 28 than at the upper surface. The sample containers 32 preferably comprise a shoulder substantially matching the frustro-conical apertures 30. To ensure easy removal of the sample containers 32 the angle of the shoulder of the sample container 32 is preferably 0.3° smaller than the angle of the frustro-conical apertures.

The holding means 28 may either be moved in a reciprocating manner, so as to agitate the contents of the sample containers 32 received in the holes 30, or the holding means 28 may simply be rotated an angle corresponding to the angle between two adjacent holes 30, so as to advance a sample container 32 to the position previously occupied by an adjacent sample container 32.

All movements of the holding means 28 are controlled by the controlling/calculating electronics 11 to which the output from the optical detectors 508 and 510 are input, and in which the voltage controlling the motor 502 is generated.

When the holding means 28 is rotated an angle corresponding to the angle between two adjacent holes 30, the holding means 28 is rotated from a position where the optical detector 508 detects an aperture 512 to a position where the optical detector 508 detects a next adjacent aperture 512.

When the motor 502, such as preferred, is a stepping motor, the control of the motor, when moving the holding means 28 in a reciprocating manner to agitate the contents of the sample containers 32, is accomplished by generating a sine-shaped

voltage such as described above, the number of periods and the frequency of the sine-shaped voltage defining the position and velocity of the holding means 28.

In two of the positions which the sample containers 32 may occupy as they are moved with the holding means 28, elevating means 524 and 526 are provided which can be made to contact and elevate the sample containers 32 in these positions (Fig. 4). The elevating means 524 and 526 are mounted on a block 528 which, via an eccentric 530, is connected to a shaft 532 of a motor 534. The elevating means 524 and 526 can be elevated from a lower position to a higher position by activating the motor 534, so as to rotate the shaft 532 an angle of 180°. When the elevating means 524 and 526 are in a lower position, an optical detector 536 detects an aperture 540 positioned close to the edge of a disc 542 fixed to the shaft 532.

The dimensions of the sample containers 32 and the container receiving means 30 and the movement of the holding means 28 are adapted so that the sample receiving part of the sample containers 32 may be substantially fixed by the elevating means 524 or 526 without subjection the sample container 32 to any influences which might tend to damage the container during agitation of the sample containers 32 which are not elevated (Fig. 7), such as mechanical stresses or collision with the neighbouring containers. This preference gives certain demands for the dimensions of the elements involved. A detailed figure of the sample container 32 is given in figure 13 wherein relevant dimensions of the preferred embodiment of the sample container 32 are given. In the preferred embodiment the angle of the shoulder of the sample container 32 is 9.7° and the angle of the frustro-conical apertures is 10.0°.

When the elevating means 524 and 526 are elevated (Fig. 6), upwardly flaring surface parts 550, formed in the block 528, are elevated. Symmetrical magnet holding arms 552 and 554 are positioned on opposite sides of the sample containers 32. The

magnet holding arms 552 and 554, each of which holds two magnets 560, are pivotable around shafts 553 and are provided with arms 555 and 557, in which frustro-conical surface parts 556 and 558, respectively, are formed. When not elevated, the surface parts 550 engage the frustro-conical surface parts 556 and 558, whereby the magnet holding arms 552 and 554 are forced away from each other, thus counteracting the magnetic attraction therebetween. When the block 528 is elevated, the conical surface parts 550, 556, and 558 disengage so that the magnet holding arms 552 and 554 are able to approach and contact, due to magnetic attraction, any sample containers 32 elevated by the elevating means 524 and 526.

The magnets 560 are positioned so that two magnets, one fixed to each of the magnet holding arms 552 and 554, contact the sample containers 32, elevated by the elevating means 524 and 526, from opposite sides (Fig. 6 and 7). Thus, when the block 528 is elevated, the magnet holding arms 552 and 554 approach due to the magnetic forces, as the magnets 560 are fixed to the magnet holding arms 552 and 554 in such an orientation so that the magnets 560 attract each other.

An additional set of opposite magnets 570 is arranged in a fixed position in a frame of the apparatus clockwise of the magnets 560 (Fig. 4). This set of magnets 570 is not able to approach and contact the sample containers 32. However, it is arranged at a sufficiently small distance from each side of a sample container 32, positioned adjacent to the magnets 570 to be able to attract any elements having magnetic properties in the sample container 32.

The elevating means 524 and 526, the additional set of magnets 570, the holding means 28, and the pipette tower 34 are positioned in relation to each other in such a way that the sample container 32, to be elevated by elevating means 526, and the neighbouring sample container 32 exposed to the additional set of magnets 570 are able to receive pipettes

held by the pipette holding segment 36, engaged in the slide member 422.

The first set of magnets 560 contacting the sample container 32, elevated by the elevating means 524, are included to reduce the processing time, and increase the efficiency of the attraction of the elements, having magnetic properties, attracted by the second set of magnets 560, as the attraction and binding of elements having magnetic properties starts one position before the position in which the magnetic fixation of the elements is actually utilized.

Fig. 3 shows a detailed schematic view of the flow of the liquid substances in an apparatus as illustrated in Fig. 1. Peristaltic pumps 102, 104, 106 and 108 pump liquid from reagent liquid containers 120, 122, 124 and 126 through liquid sensors 116 and lines 170, 172, 174 and 176 to dosing pipettes 38, held in pipette holding segments 350, 352, 354 and 356, respectively.

A pump 110 pumps liquid from a flushing liquid container 128 through a line 180 and, depending on a valve 140, through a line 182 to a liquid supply line 210 (Fig. 10) in withdrawal and flushing pipette 40 held by the pipette holding segment 356, or through a line 186 to a valve 142 which controls the flow of the liquid through lines 184 and 188 to liquid supply lines 210 in withdrawal and flushing pipettes 40 held by the pipette holding segments 354 or 352 respectively.

A pump 112 pumps liquid from a valve 144, through a line 190 to a liquid container 130. This liquid is, depending on the valve 144, pumped through a line 192 and a liquid removing part 212 (Fig. 10) of a withdrawal and flushing pipette 40 held by the pipette holding segment 354, or through a line 194 and a liquid removing part 212 of a withdrawal and flushing pipette 40 held by the pipette holding segment 352.

A pump 114 pumps liquid from a liquid removing part 212 of a withdrawal and flushing pipette 40, held by the pipette holding segment 356, to the liquid container 130 through a line 200, a measuring cuvette 300, and a line 202 to the liquid container 130.

During operation, the liquid sensors 116 sense the presence of liquid in the lines 170, 172, 174, 176, 180, 190, 192, 200 and 202. Absence of liquid in one of these lines may lead to incorrect results as liquid may not be dosed when intended. A "no-liquid signal" from a liquid sensor 116 may give rise to the apparatus 10 informing the operator which sample containers 32 have not received all relevant liquid or from which sample containers 32 not all relevant liquid has been removed, and therefore which measurements should be repeated.

The optical measuring module 42 (Fig. 3) comprises a light emitter 302, preferably a halogen lamp, a collimating lens 304 transforming part of the light emitted from the light emitter 302 into a parallel beam of light, and a focusing lens 310 focusing part of the collimated light onto the measuring cuvette 300. The lenses 304 and 310 are preferably chosen so that the light, focused inside the measuring cuvette 300, forms an image having the same size as a light emitting element 303 in the light emitter 302. While the light is collimated, it is launched through a heat filter 306 removing IR light emitted from the light emitter 302. The IR light might otherwise destroy a band pass filter 308 which transmits light in a predetermined waveband able to excite a fluorophore in a sample contained in the measuring cuvette 300.

When the a fluorophore in the sample contained in the measuring cuvette 300 is exited due to the illumination, fluorescent substances in the sample will emit fluorescence having a wavelength longer than the exciting light transmitted through the filter 308.

Part of this fluorescence is collimated by a collimating lens 312 and focused on to a light detector 318 by a focusing lens 317. While the fluorescence is collimated, it is launched through filters 314 and 316 together forming a band pass filter removing light having a wavelength not being in a wavelength region of the fluorescence emitted from the exited sample.

The collimating and focusing lenses 312 and 317 are preferably selected so that the fluorescence launched on to the light detector 318 has the same size as the light emitting element 303 in the light emitter 302. The light detector 318 is preferably selected so that the light sensitive surface (not shown) of the detector 318 has the same size as the light emitting element 303. The above-mentioned selections are performed to increase the signal to noise ratio of the optical measurement.

As the wavelength composition of the light emitted from the light emitter 302 may vary slightly due to a number of factors e.g. the voltage supplied to the light emitter 302 or the temperature or the age of the light emitter 302, a reference light detector 320 is positioned close to the measuring cuvette 300. The reference light detector 320 is positioned in a way so that it receives a small amount of the light launched on to the measuring cuvette 300. In this way, variations in the amount of exciting light launched onto the measuring cuvette 300 may be compensated for when determining the amount of fluorescence emitted from the sample contained in the measuring cuvette 300.

The determination of the concentration of a substance to be determined is performed in the controlling/calculating electronics 11 based on a calibration of the optical measurement and the amount of liquids added to the sample containers 32.

The solid black arrows in Fig. 3 show the direction of controlling/monitoring signals in the apparatus. It is seen that all pumps, valves, and motors are controlled by the controlling/calculating module 12, that the optical detectors, the liquid sensors and the light detectors generate signals transmitted to the controlling/calculating module 12, and that the interface 16 exchanges information with the controlling/calculating module 12.

In operation, the apparatus 10, may be used for e.g. measuring the contents of a fluorescent agent produced by an immunological or another process taking place in the sample containers.

An ELISA analysis based on the so-called "sandwich-technique" using two different monoclonal antibodies against - in this specific case - *Salmonella* antigens may be performed as described in the following (see Example 1, below, for an explanation of the terms **Wash**, **Calibrator**, **Conjugate**, **Substrate**, **Immunospheres** and **Stop**); the antibodies used react slightly with the native flagella, but strongly with a heat-treated extract of *Salmonella*. Therefore, the samples are preferably heat-treated at 100°C for 15 minutes before performing the ELISA. This heat-treatment kills/inactivates the *Salmonella* and other pathogenic bacteria. The ELISA is able to detect 10^5 cells per ml, and the samples should therefore comprise at least this number of cells per ml.

The automated ELISA analysis on an apparatus according to the invention typically takes approximately 75 minutes, but can be performed manually.

PROCEDURE

- (step 1): one test tube with **Wash** (negative control) and one test tube with 500 µl **Calibrator** (a heat-treated suspension of *Salmonella* typhimurium diluted to a concentration of approximately 10^6 cells per ml) are placed

in a sample disc together with test tubes containing 500 µl heat-treated samples. The samples are warmed up to 35°C and the analysis starts.

- (step 2): 90 µl **Immunospheres** are added to each sample and incubated for 12½ minutes. *Salmonella* antigens present in the sample will react with antibody and form immunecomplexes on the surface of the **Immunospheres**. The total surface area of the microspheres is very large, and this allows a fast and efficient binding between antibodies and antigens to take place.

- (step 3): Non-bound material is washed away with **Wash** while the **Immunospheres** are retained in the side of the test tube by magnetic force. 130 µl **Conjugate** is then added and incubated 12½ minutes. The conjugated antibody binds to the immunosphere-antigen complex. This creates a "sandwich" with *Salmonella* antigens as the "filling".

- (step 4): Non-bound conjugated antibody (**Conjugate**) is washed away with **Wash**.

- (step 5): 200 µl **Substrate** is added and incubated 12½ minutes. The enzyme bound to the particles will split the substrate to, inter alia, 4-methylumbelliferyl, a fluorescent compound which is measured by a fluorescence detector (excitation 365 nm, emission 450 nm).

- (step 6): The enzyme reaction is stopped by adding 200 µl **Stop**.

- (step 7): The concentration of 4-methylumbelliferyl is now measured by the detector and the concentration is proportional to the amount of *Salmonella* antigen present in the sample. The fluorescence signal for the individual sample is compared to a cut-off value, defined as 25% of the calibrator signal. Samples with a signal higher than or equal

to the cut-off value are considered positive. Samples with a signal below the cut-off value are considered negative.

Magnetic particles to which antibodies are bound may be made in a manner known per se by coating paramagnetic particles, such as DYNABEADS® with the antibody in question. While, in the above example of an immunological process, the element bound to the magnetic elements is an antibody, it will, of course, as already mentioned, also be possible to have an antigen bound to the magnetic elements, etc. in cases where the component to be determined is an antibody.

The following section describes the manner of operation of the apparatus 10 according to the invention in connection with the performance of an immunochemical determination of the type described above.

After turning on the apparatus 10, the apparatus performs a self-test of the controlling/calculating electronics 11 comprised in the controlling/calculating module 12.

The starting position, position 1, is the position of the container receiving means 30 in the holding means 28 which is positioned directly above the elevating means 526 when the optical detector 510 detects the aperture 514. From this position the positions for the sample containers 32 are numbered in counter clockwise direction.

Step 1: adding the samples

The samples are added to the sample containers in positions 4 to 30, which is the total number of container receiving means 30 in the holding means 28 in a preferred embodiment of the apparatus according to the invention.

Then sample containers 32 are placed in the container receiving means 30, and an amount of **Calibrator** is manually transferred to a sample container 32 in position three, seen

counter clockwise from a starting position (position 1) of the holding means 28.

The **Calibrator** is preferably a liquid containing a known concentration of the substance to be determined. This liquid will then be treated as a real sample, and as the **Calibrator** will be transferred to the measuring cuvette 300, the measured fluorescence, corresponding to the known concentration of the substance to be determined, may then be used for calibrating the optical measurement. This calibration will be described below.

Now the sample containers contain:

Position No. 1	Empty
Position No. 2	Empty
Position No. 3	Calibrator liquid
Position No. 4	Sample
.	
.	
Position No. 30	Sample

Before starting the e.g. immunological reactions by pumping liquid from the liquid container 120 to the sample containers 32 containing the samples (positions 4 to 30), the contents of the container 120 are agitated to ensure a homogeneous composition of the contents.

The agitation of the contents of the container 120 is performed by activating a motor 132, connected to a suction pipe 134 in the liquid container 120 by an eccentric 136 which moves the suction pipe 136 so as to agitate the liquid in the liquid container 120.

After this, the apparatus performs a "priming", where the lines leading liquids to the dosing pipettes 38 are filled with liquid.

To ensure that the lines 170, 172, 174, and 176 have been filled with liquid, small volumes of the corresponding liquids are dosed into the sample container 32 in position 1 in the holding means 28.

A detailed description of the dosing and rotation of the holding means 28, necessary for the relative positioning of the sample container 32 and the dosing pipette 38, is given below.

During the priming of the line 170, the corresponding liquid sensor 116, of a type known per se, is used for ensuring that when the pump 102 is activated, liquid is dosed into the sample container 32.

After liquid is detected by the liquid sensor 116, the pump 102 is activated to pump a predefined volume of liquid, hereby ensuring that liquid is dosed into the sample container 32 in position 1 in the holding means 28.

A similar procedure is performed for priming the liquids in the liquid containers 122, 124, and 126.

After the priming of these lines, a volume of the liquid from liquid container 128 is dosed into the empty sample container 32 in position 2 in the holding means 28, this liquid constituting a blind sample.

This blind sample, which does not contain any of the substance to be determined, is treated as a normal sample. Due to this, the blind sample may be used for supervising the reaction and compensating for a "blind level".

To improve the precision and repeatability of the measurements, the processes preferably take place at a controlled temperature. Therefore, after the self test, the controlling/calculating electronics 11 activates two fans 382 and three sets comprising two heating elements 380 and a tempe-

rature sensor 384, positioned in the sample handling and measuring module 14. The temperature sensors 384 transmit signals to the controlling/calculating module 12 so that the desired temperature in the sample handling and measuring module 14 may be obtained and maintained.

Now the apparatus 10 is ready to start the immunological process in the sample containers 32 holding the samples, to perform the UV-measurement on the fluorescent agent of the process, and to determine the amount of this fluorescent agent in the sample.

Step 2, adding magnetic particles

In this step the **Immunospheres**, having magnetic properties, are added to the samples to attach to the substance to be determined.

Now the first sample container holding a "sample" (position number 2 in the holding means 28 as the **Calibrator** and the blind sample are treated as samples) is, by activating the motor 502, positioned in the position where it is exposed to the additional set of magnets 570.

Then the sample containers 32 positioned above the elevating means 524 and 526 are elevated by activating the motor 534, rotating the shaft 532 an angle of 180°, whereby the elevating means 526 are elevated and the magnet holding arms 552 and 554 approach and contact the sample containers 32 in question.

Then the top part 35 of the pipette tower 34 is rotated to place the pipette holding segment 350, holding the dosing pipette 38, in engagement with the slide member 422, which is then positioned in the intermediate position.

Then the pipette 38, held by the pipette holding segment 350, is lowered into the sample container 32 exposed to the

additional set of magnets 570, whereafter the pump 102 is activated, and a volume of the liquid from liquid container 120 is added to the sample container 32 in question.

After this, to ensure a the highest precision in the dosing of the liquid, the pipette 38, held by the pipette holding segment 350, is moved up to a higher position A (Fig. 12 and example 2), whereafter the slide member 422 is given a velocity down toward the sample container 32. At a position B (Fig. 12), higher than the surface of the sample in the sample container 32, this velocity is reversed, giving the tip of the dosing pipette 38 an acceleration in an upward direction. This gives the liquid on the tip of the dosing pipette 38 an acceleration in a downward direction in relation to the pipette tip.

However, the force F_a applied to the drop may have a size so large that the drop breaks up. Due to the direction of the applied force F_a, the released drop will fall from the pipette 38 to be added to the sample positioned below the pipette 38.

The critical volume of a drop, being the maximum volume that a drop may have without breaking due to the applied force, depends on the size of the applied force F_a.

In this way the volume interval of drops remaining on the tip of the pipette 38 after dosing is substantially reduced, resulting in an increased precision of the dosing and an increased repeatability of the dosing precision of the apparatus 10 according to the invention.

The movement of the slide member 422 is achieved by activating the motor 424 and controlling the motor on the basis on a signal generated by the controlling/calculating electronics 11.

This jerk movement is repeated; this time the velocity of the pipette 38, held by the pipette holding segment 350, is reversed at a position C higher than position B. Then the slide member 422 is again positioned in the intermediate position.

A translation of the signals transferred to the motor 424 is shown in figure 11. Here the theoretical movement pattern of the dosing pipette 38 is shown where the y-axis is the position (height) of the tip of the pipette 38, and the x-axis is the time. A further explanation of this jerk-movement is given in Example 2.

It will be understood that the above-described jerk movement to ensure the highest precision in the dosing is performed in connection with all of the dosing operations described in the following description of this preferred embodiment.

Then the elevating means 524 and 526 are lowered, and the holding means 28 is rotated clockwise one position.

Now the elevating means 524 and 526 are elevated and the contents of the sample containers 32, not elevated (Fig. 5), are agitated by activating the motor 502, such as will be described below.

Figure 5 shows a sample container 32 which is not elevated. This sample container 32 is placed in a "close fit" in which the sample container 32 substantially follows the movement of the holding means 28.

Figure 6 shows an elevated sample container 32 contacted by the magnets 560. It is seen that the sample container 32 is no longer in the "close fit" seen in fig. 5, the sample container 32 is able to move relative to the holding means 28.

Figure 7 shows an elevated sample container 32 during agitation of the samples held by the sample containers 32 which are not elevated. It is seen that the lower, sample-containing part of the sample container 32 is contacted and substantially fixed by the magnets 560 and the elevating means 524 or 526.

When moving the holding means 28 in an oscillating manner, the elevated sample container 32 is able to rotate about an axis positioned in the liquid sample held by the sample container 32 in such a way that the top part of the sample container 32 may substantially follow the movement of the holding means, while the lower part of the sample container 32 may remain substantially fixed in relation to the magnets 560 and the elevating means 524 or 526.

Due to the fact that the rotation of the sample container 32 takes place about an axis positioned in the sample held by the sample container 32, the liquid is only slightly agitated (Fig. 7).

As the agitation of the elevated samples is substantially prevented, this feature enables the magnets to attract and hold any elements having magnetic properties, while other samples in the holding means are agitated. Due to this substantial prevention of the agitation of a sample in an elevated container 32, it will also be possible, if desired, to add liquid to or withdraw liquid from a thus elevated container 32 while the samples in the other containers 32 are agitated.

The agitation of the samples in the containers 32 is preferably performed in such a way that the holding means 28 is given a larger velocity in one direction than in the other direction.

The reason for this choice is the fact that the force acting on the liquid in the sample containers 32 is the centrifugal

force. This force depends on the square of the angular velocity of the holding means 28, which means that if the velocity of the holding means 28 in one direction is, e.g., twice the velocity of the other direction, the force acting on the liquid is four times higher in one direction than in the other.

As the angular velocity is larger for the parts of the liquid in the sample containers 32 which are closest to the outer edge of the holding means 28, those parts of the liquid will respond strongest to the movement of the holding means 28.

This gives the liquid in the sample containers 32 a circular movement in the sample containers 32 (Fig. 8) which circular movement is preferred for the optimum mixing of the contents of the sample containers 32. By this movement, a very efficient mixing and distribution of the contents of the sample container 32 is ensured.

As described above, the motor 302 is preferably a stepping motor which may be controlled by a sine-shaped voltage. To ensure that the holding means 28, after one agitation period, returns to the starting position, care is taken that the motor 302 receives the same number of periods of the sine-shaped voltage in the two directions. When giving the holding means 28 e.g. twice the velocity in one direction than in the other, the frequency of the sine-shaped voltage is twice as high in the one direction than in the other.

The position of the holding means 28 during agitation, as a function of time, may be seen from Fig. 9, where the vertical dashed lines show the periods of the agitation cycle of an apparatus according to the invention. In Fig. 9, the y-axis represents the position of the holding means 28 and the x-axis represents the time.

The time passing between lines A and B corresponds to the time used for the faster movement in one direction. The time

passing between lines B and C corresponds to the time used for the slower movement in the other direction of the holding means 28. From Fig. 9, it can be seen that the time used for the slower movement is approximately 3.4 times larger than the time used for the faster movement. It can also be seen that the acceleration in the faster movement is higher than the acceleration in the slower movement.

Step 2 is repeated once for each sample holding sample container 32 in the holding means 28.

Then the slide member 422 is positioned in the upper position whereby the catch (not shown) disengages the top part 35 which is now able to rotate. Then the top part 35 is rotated to its starting position by activating the motor 404 and monitoring the rotation of the top part 35 by means of the detectors 408 and 414.

Step 3

In this step, the magnets 560, fixed to the magnetic holding arms 552 and 554 brought into contact with the sample containers 32 when elevated, are utilized to attract and hold the magnetic particles, to which the substance to be determined is bound, while the liquid in the sample containers 32 is withdrawn, whereupon flushing is performed. After this, the second substance is added to the sample containers 32.

Now the first sample, to receive liquid in step 2 (position 2), has been thoroughly agitated, and the reaction, in which the substance to be determined binds to the magnetic particles, has been allowed to proceed for a predetermined period of time. This sample container is now, by activation of the motor 502, positioned in the position directly above the elevating means 524.

The elevating means 524 and 526 are again elevated, contacting the sample containers 32 positioned directly above the elevating means 524 and 526, and the magnets 560 contacting the sample containers 32 attract and hold the magnetic particles present in the sample containers 32.

The top part 35 of the pipette tower 34 is rotated so that the pipette holding segment 352 is brought into engagement with the slide member 422. This pipette holding segment 352 holds two pipettes: a withdrawal and flushing pipette 40 and a dosing pipette 38 positioned so that the withdrawal and flushing pipette 40 is lowered into the sample container 32 elevated by the elevating means 526, so that, in the way of rotation of the sample holding means 28, the liquid in the sample container is withdrawn and the sample container is flushed, and then, after a rotation of one step by the sample holding means 28, an amount of the liquid from the liquid container 122 may be dosed from pipette 38, held by the pipette holding means 352.

The withdrawal of liquid from and the flushing of a sample container 32 is performed by lowering the withdrawal and flushing pipette 40 to a position in which it is able to remove substantially all liquid from the sample container 32. After ensuring that the valve 144 transfers liquid from the lowered withdrawal and flushing pipette 40 to the liquid container 130, the pump 112 is activated whereby substantially all liquid is removed from the sample container 32.

Due to the magnets attracting the magnetic particles, the substrate to be determined is not removed from the sample container 32.

When the pump 112 has been activated for a given period of time, it is turned off and the pump 110 is activated to introduce liquid from the liquid container 128 into the sample container 32. The valves are, of course, controlled so

that the liquid pumped from the liquid container 128 is in fact transferred to the sample container 32 in question.

The liquid exits the withdrawal and flushing pipette 40 from apertures 214 positioned in positions higher than the surface of the liquid previously present in the sample container 32. In this way, the liquid runs down the outer surface of the withdrawal and flushing pipette 40 so that the part of outer surface which was in contact with the sample in the sample container 32 is flushed. As the flushing liquid is dosed while the pipette tip is positioned in the sample container 32, both the withdrawal and flushing pipette 40 and the sample container 32 are flushed in this action.

When a given volume, preferably larger than the amount of liquid removed, of the liquid from liquid container 128 has been is introduced in the sample container 32, the pump 110 is turned of.

To obtain a satisfactory degree of flushing, liquid is again withdrawn from the sample container 32, whereupon liquid from the liquid container 128 is again introduced into the container.

Then substantially all liquid is again withdrawn by the withdrawal and flushing pipette 40 and, after rotation of one position of the holding means 28, an amount of the **Conjugate** is dosed from the liquid container 122 by activating the pump 104 while the next container (one position higher) is emptied and flushed by the withdrawal and flushing pipette 40 held by the pipette holding means 352.

After addition of the **Conjugate**, due to the attraction to the magnets, the magnetic particles may tend to stick to the sides of the sample container 32 for approximately two positions even though the sample container is agitated. After this, the agitation of the sample container 32 will resuspend

the magnetic particles, and the substance bound to which may then react with the added **Conjugate**.

This step is repeated until all sample-containing sample containers 32 have been subjected to withdrawal of liquid and flushing and a volume of the **Conjugate** contained in liquid container 122 has been added thereto.

Step 4, flushing

As the **Conjugate** added in step 3 is typically added in an amount larger than the amount actually binding to the substance to be determined, some of the dosed **Conjugate** is unbound.

This unbound **Conjugate** has to be removed from the sample containers 32 as the next reaction is not able to distinguish between bound and unbound elements. In the next reaction, the **Conjugate** chemically splits the **Substrate**, thus forming a fluorescent product. The amount of fluorescent product generated in this reaction depends on the total amount of the **Conjugate**, bound as well as (undesired, and to be removed) unbound.

Withdrawal of liquid and flushing is performed as described above using the withdrawal and flushing pipette 40 held by the pipette holding segment 354.

As this withdrawal and flushing may not effectively remove unbound **Conjugate** trapped between the attracted elements in the sample container, a third amount of flushing liquid is dosed after the second dose of flushing liquid has been withdrawn, and the holding means 28 is rotated to flush the next sample container 32.

To ensure removal of substantially all of the unbound **Conjugate**, this flushing step is repeated after the holding means 28 has performed a full circle where the contents of

the sample containers 32 have been resuspended and mixed with the third amount of flushing liquid. Now substantially no unbound, previously trapped, part of the **Conjugate** will be trapped when the magnetic particles are again attracted by the magnets and the flushing is repeated, whereby substantially all of the unbound **Conjugate** may be flushed away.

Step 5

In this step, the **Substrate** to be chemically activated or split by the **Conjugate** is added.

A pipette holding segment 354, holding two pipettes 40 and 38 in the same configuration as in step 2, is lowered to remove substantially all liquid from the sample containers 32, while holding back the magnetic particles.

After rotating the holding means 28, a volume of the **Substrate** contained in container 124 is added while the next sample container 32 is subjected to withdrawal of liquid and flushing.

Step 6

In this step, a pipette holding segment 356 is used in which two pipettes 38 and 40 are positioned in such a manner that the sample containers 32 first receive an amount of liquid from the liquid container 126 (**Stop**), so as to stop the reaction started in step 3, from the dosing pipette 38, whereafter, after rotating the holding means 28 one position, step 7 is performed wherein the optical measurement is performed.

Step 7

The last step is the determination of the fluorescence induced in the sample. The amount of fluorescence depends on

the concentration of the substance to be determined in the original samples.

After dosing the liquid in step 6, the elevating means 524 or 526 are lowered, the holding means is rotated one position, and the elevating means 524 and 526 are again elevated. Then the samples which are not elevated, including the sample which has just received the liquid in step 6, are agitated. Due to the magnets 570 and the fact that the magnetic particles are not resuspended until they have been agitated two times, the magnetic particles which were previously attracted by the magnets 560 are not resuspended while the sample is agitated. This agitation ensures that the liquid dosed in step 6 actually stops the reaction taking place in the sample container 32, and that the liquid to be transferred to the measuring cuvette 300 is homogeneous, which means that it does not contain any substantial amount of the magnetic particles attracted to the magnets 570.

The un-attracted contents of the sample container 32 are transferred to the measuring cuvette 300 through a withdrawal and flushing pipette 40 held in the pipette holding segment 356.

Removing the liquid from the sample container 32 and transferring this liquid to the measuring cuvette 300 is performed by activating the pump 114 which removes liquid from the sample containers 32 through line 200 to the measuring cuvette 300 and further through line 202 to the liquid container 130.

Calibration of the UV measurement

As described above, the samples contained in the sample containers 32 in positions 2 and 3 in the holding means 28 are, in fact, a blind sample and a calibrator liquid, respectively, both samples containing known concentrations of the substance to be determined.

For calibration of the UV-measurement, these liquids are, in turn, transferred to the measuring cuvette 300 in the optical measuring module 42 where the light emitter 302 is activated.

The pump 112 pumps liquid through line 200 into the measuring cuvette 300. When part of the sample has been pumped through the measuring cuvette 300, the pump 112 is stopped so that the measurement is not performed on the first liquid to enter the measuring cuvette 300. This is to ensure that the liquid present in the measuring cuvette is not contaminated or diluted by any liquid present in the flushing pipette 40, line 200, or the measuring cuvette 300.

The intensity of the light emitted from the light emitter 302 is adjusted by adjusting the voltage fed to the light emitter 302. The light launched on to the measuring cuvette 300 excites the calibrator liquid in the cuvette 300 so that fluorescence is detected by the light detector 318. The intensity of the light emitted from the light emitter 302 is adjusted so that the light detector 318 detects a predetermined intensity of fluorescence. The voltage fed to the light emitter 302 will now be held at a constant value as the intensity of the light launched on to the measuring cuvette 300 varies only slightly, and may be compensated for as described below.

A fraction of the light launched on to the measuring cuvette 300 is detected by the reference light detector 320. When the detector 318 detects the predetermined intensity of fluorescence, the output (reference output) from the detector 320 is stored in a memory (not shown) in the controlling/calculating module 12.

This reference output value of the detector 320 is later on used in the determination of the fluorescence emitted from the sample contained in the measuring cuvette 300, for compensating for slightly deviating intensities of exciting light launched on to the measuring cuvette 300.

Now the UV-measurement is calibrated, and the sample container 32 and the withdrawal and flushing pipette 40, held in the pipette holding segment 356, are flushed by activating the pump 112 and ensuring that the valve 144 transfers liquid from the liquid container 130 through line 182 to the liquid supply line 212 of the withdrawal and flushing pipette 40 held by the pipette holding segment 356.

After dosing an amount of liquid, the pump 112 is stopped, and the pump 114 is activated to remove the liquid from the sample container 32 through lines 200, 202 and measuring cuvette 300, so as to also flush these elements.

The flushing steps are repeated to obtain a sufficient degree of flushing of the pipette 40, the sample container 32, line 200, and the measuring cuvette 300.

Then the motor 424 is activated, as described above, to elevate the slide member 422 and the pipette holding segment 356 holding pipettes 38 and 40, to the intermediate position.

Performing the optical measurement

The UV-measurement performed on the samples is performed in the same manner as the calibration.

Part of the fluorescence emitted from the excited sample and part of the light launched on to the measuring cuvette 300 are measured by the light detector 318 and the reference detector 320 respectively.

On the basis of the signals from the two detectors 318 and 320, the controlling/calculating module 12; on the background of the calibration, is able to determine the amount of the fluorescent agent, and thereby the amount of the substance to be determined, in the sample.

After this, the rest of the sample is removed from the sample container 32 by again activating the pump 114. And the sample container 32, the withdrawal and flushing pipette 42, line 200, and the measuring cuvette 300 are flushed as described above.

When the UV-measurement is performed, a value corresponding to the amount of the substance to be determined in the original sample is calculated. This value is compared to a selectable cut-off value, and samples having values higher than this cut-off value may contain high levels of the substance to be determined.

Rinsing the apparatus

After a measuring series the apparatus should be rinsed.

The rinsing is performed by removing the liquid containers 120, 122, 124, and 126, and inserting a container (not shown) containing a rinse liquid. This container has a shape so that the pumps 102, 104, 106, and 108 may pump liquid from the container (not shown) through lines 170, 172, 174, and 176 to the dosing pipettes 38 held by the pipette holding segments 350, 352, 354, and 356.

Some of this liquid is then pumped into empty sample containers 32 in position 1 and 2 in the holding means 28. This liquid is then sucked into lines 192, 194, 190, 200, and 202 through the liquid removing parts 212 of the withdrawal and flushing pipettes 40 held in the pipette holding segments 352, 354, and 356. This is accomplished by positioning the withdrawal and flushing pipettes 40 in the sample containers 32 holding the rinse liquid after turn, and filling the lines with the rinse liquid.

The liquid is allowed to react for a period of time whereafter all lines are emptied into the container 130.

Now the apparatus is rinsed and ready for a new measurement.

Other types of measurements

Due to the increased dosing accuracy, the agitation, the temperature control, and low cross-contamination, this apparatus is excellently suited also for other types of chemical or immunological measurements.

When starting e.g. a chemical reaction and performing a measurement on the resulting product(s), the precision of the measurement depends both on the precision when dosing the reactants, and the conditions under which the reaction takes place.

As an example, determination of enzymes may also be performed on an apparatus according to the present invention. A simple determination of an enzyme may comprise the following steps:

- Step 1 will be conceptually of the same type as previously described above in connection with the determination of *Salmonella* antigens, and will involve adding the samples containing the enzyme to be determined to the sample containers and preparing the apparatus for the measurement.
- In step 2, a substance or species reacting with the enzyme to be determined is added. The product of this reaction may be measurable by, e.g., an optical measurement.
- Step 3 will then be the determination of the product(s), enabling a calculation of the concentration of the enzyme in the original sample to be determined.

Step 2 may be replaced by more complicated reactions, e.g. reactions involving more reactants, but the pattern of the determination will be as described.

EXAMPLE 1

When a preferred embodiment of an apparatus according to the invention is used for an ELISA type determination of flagellin e.g. from *Salmonella* bacteria, the liquids contained in the liquid containers are:

Liquid container	Liquid
120	**Immunospheres** (Dynabead® (a registered trademark owned by Dynal, Norway), 0.6 mg/ml) coated with monoclonal antibody against a *Salmonella* common flagellar antigen
122	**Conjugate** Salmonella monoclonal antibody conjugated to the enzyme β-galactosidase (2 μg/ml)
124	**Substrate** 4-methylumbelliferyl-β-galactoside, 100 μM, 1 mM $MgCl_2$ in 0.7% dimethylformamide
126	**Stop** Glycine buffer, 0.1 M, pH 10.5
128	**Wash** PBS (phosphate buffered saline, 0.9% NaCl) + 0.1% Triton-X100, Ph 7.4

The **Calibrator** is a dissolved heat extract of S. typhimurium. Fully grown culture in M-Bouillon (DIFCO), dissolved approx. 1:3,000 in M-Boullion.

The optical elements are:

Light emitter (302):	10 W halogen bulb
Heat filter (306):	Melles griot KG1
Filter (308):	BP 365, 365 nm ± 10 nm
Filter (314):	LWP 420, 420 nm ± 3 nm
Filter (316):	SWPBL720, 720 nm ± 10 nm

Reference detector (320):	Hamamatsu S1133-05
Light detector (318):	Hamamatsu S1227-16BR
Measuring cuvette (300):	Glass tube, 1.1 mm light path, 10 µl volume.

The lenses (304, 310, 312, and 317) are aspheric condenser lenses having f=15.5 mm purchased from Spindler Hoyer.

This setup gives

365 nm light on to the cuvette 300:	0.5 - 8 µW
450 nm fluorescence from the cuvette:	11.3 pW - 113 nW
450 nm fluorescence on to the detector:	6.8 pW - 68 nW

This apparatus is able to measure fluorescence varying 4 decades. A standard, related to the fluorescent substance used in the measurement of flagellin e.g. from Salmonella bacteria, is representative of the concentration of the fluorescent element (4-Methylumbelliferyl-ß-D-galactoside, abbreviated: 4-MU) in the sample. A preferred embodiment of an apparatus according to the invention is able to measure 2.5 nmol - 25 µmol 4-MU.

When the calibration of the optical measurement has been performed, fluorescence corresponding to a standard value, typically approx. 2500 nmol 4-MU or more, is considered "positive", which means that there is a possibility that the original sample has a too high content of flagellin. The operator should confirm this diagnosis by a standard method of determining the concentration of flagellin in the original sample.

However, this apparatus is in fact able to perform quite precise measurements as it has a measuring range of 4 decades, (2.5 nmol - 25 µmol 4-MU) and a signal to noise ratio of 6.8:1 at 2.5 nmol 4-MU.

The relative standard deviation of the light detection of the detector unit is less than 1%, typically in the order of 0.5%.

The calibration of the measurement is typically performed so that the measurement is linear for typical values and slightly nonlinear for the highest values of fluorescence where there is no doubt that the original sample has a too high content of flagellin.

The error due to non-linearity: 0-5% at 25 µmol 4-MU

Due to the efficient flushing procedure, the cross contamination is as low as 0.01%.

A determination of flagellin in a sample is performed as described above:

Step	pipette	pipette holding segment	action
1			Add 500 µl **Calibrator** to the sample container in position 3, add samples in containers 4-30, and start the measurement.
2	38	350	Dose **Immunospheres**, 90 µl. Rotate the holding means and agitate the samples not elevated.
3	40	352	Remove substantially all liquid.
	-	-	Dose 600 µl **Wash**.
	-	-	Remove substantially all liquid.
	-	-	Dose 600 µl **Wash**.
	-	-	Remove substantially all liquid. Rotate the holding means and agitate the samples not elevated.
	38	-	Dose 130 µl **Conjugate**.

				Rotate the holding means and agitate the samples not elevated.
	4	40	-	Remove substantially all liquid.
		-	-	Dose 250 µl **Wash**.
		-	-	Remove substantially all liquid.
		-	-	Dose 250 µl **Wash**.
				Rotate the holding means and agitate the samples not elevated.
	Repeat step 4			
	5	40	354	Remove substantially all liquid.
				Rotate the holding means and agitate the samples not elevated.
		38	-	Dose 200 µl **Substrate**.
				Rotate the holding means and agitate the samples not elevated.
	6	38	356	Dose of 200 µl **Stop**.
				Rotate the holding means and agitate the samples not elevated.
	7	40	356	Remove substantially all liquid through the measuring cuvette 300.
		-	-	Determine the UV-induced fluorescence.
				Dose 200 µl **Wash**.
		-	-	Remove substantially all liquid through the measuring cuvette 300.
		-	-	Dose 200 µl **Wash**.
		-	-	Remove substantially all liquid through the measuring cuvette 300.
		-	-	Dose 200 µl **Wash**.
		-	-	Remove substantially all liquid through the measuring cuvette 300.

The relative standard deviation obtained when performing a measurement as described above wherein all samples have been

replaced with calibrator liquid is less than 3%, typically 1-2%.

EXAMPLE 2

A test has been made wherein the dosing accuracy of a pipette having an inner diameter of 0.56 mm and a wall thickness of 0.25 mm without using the jerk-movement is compared to the accuracy of a pipette having an inner diameter of 0.56 mm and a wall thickness of 0.15 mm using the jerk-movement.

In this example, an amount of the liquid containing the particles having magnetic properties, used in the immunological measurements, is dosed into a number of sample containers. The weight of the sample containers is measured before and after the addition of the liquid whereby the dosed amount may be determined.

No jerk-movement

dosed amount (g)	deviation (%)
0.0996	-0.9
0.1016	1.1
0.0985	-2.0
0.0997	-0.8
0.1033	2.8

Mean value: 0.10054 g
Deviation: -2.0 - 2.8%
Relative standard deviation: 1.7%

With jerk-movement

dosed amount (g)	deviation (%)
0.1057	0.00

0.1056	-0.01
0.1058	0.01
0.1057	0.00
0.1057	0.00
0.1056	-0.01
0.1057	0.00
0.1062	0.05
0.1057	0.00
0.1056	-0.01

Mean value: 0.1057 g
Deviation: -0.01 - 0.05%
Relative standard deviation: 0.16%

These results indicate that the jerk-movement is an extremely efficient way of increasing the accuracy of dosing.

CLAIMS

1. An apparatus for performing unit operations involving the handling of liquid samples, the apparatus comprising:

a number of sample containers, each having a portion in which a liquid sample can be contained,

means for adding liquid to the sample containers, and means for removing liquid from the sample containers,

the apparatus showing at least one of the following features a)-d):

a) holding means for holding the sample containers, the holding means having container-receiving means adapted to receive each container in male-female relationship with the container-receiving means, and means for oscillatingly moving the holding means and thereby transferring movement from the holding means to the sample container to agitate liquid in the sample container received by the container-receiving means,

b) the means for adding liquid to the sample containers or removing liquid from the sample container comprise a plurality of pipettes arranged in pipette holding and moving means adapted to hold the pipettes and to move each individual pipette to a position relative to a sample container in which the pipette is capable of exerting its function of dosing liquid to the container or remove liquid from the container

c) the dosing pipette means are movable by pipette moving means which are adapted to confer to the pipette means an upward acceleration at the end of a pipetting procedure so as to release from the pipette tip any part of any liquid drop in excess of an amount determined by the upward acceleration,

d) the means for removing liquid from the sample containers comprise suction pipette means for sucking the liquid from the containers combined with flushing liquid supply means adapted to supply flushing liquid through an outer surface part of the suction pipette means.

2. An apparatus according to claim 1, adapted for quantitative or qualitative determination of a component in liquid samples by performing, on individual liquid samples, a reaction which can be detected by a chemical or physical measurement or which directly or indirectly results in a product which is representative of the component and which can be detected by a chemical or physical measurement, the apparatus further comprising measuring means adapted to perform said chemical or physical detection of the reaction or the product resulting from the reaction.

3. An apparatus according to claim 2, wherein said measurement is an optical measurement and said measuring means is an optical measuring means adapted to perform optical detection of the reaction or the product resulting from the reaction.

4. An apparatus according to any one of claims 1-3 and comprising holding means a), further comprising means for moving at least one container axially in relation to the corresponding receiving means between a first position, in which the sample container is received in the holding means with a relatively close fit, and a second position in which the sample receiving part of the container may be moved transversely in relation to the receiving means.

5. An apparatus according to any one of claims 1-4, wherein the outer surface of each container defines a shoulder between its opposite ends, and each of the receiving means of the holding means defines a support surface for engaging with the shoulder of the container.

6. An apparatus according to claim 5, wherein the holding means is a plate-like member with a plurality of apertures for receiving the sample containers.

7. An apparatus according to claim 6, wherein each aperture has a substantially circular outline, the diameter of the aperture at the lower surface of the holding means being smaller than at the upper surface thereof.

8. An apparatus according to claim 7, wherein each aperture is at least partly defined by a frustro-conical surface part, the shoulder of each of the containers defining a substantially complementary frustro-conical surface part being in abutting engagement with the first mentioned frustro-conical surface part in the first position of the container.

9. An apparatus according to claim 8, wherein the top angle of the frustro-conical surface parts are within the range of 3°-18°, preferably 6°-13°, and more preferably within 9°-11°.

10. An apparatus according to claim 9, wherein the top angle of the conical surface part of the container is smaller, preferably 0.1°-1° smaller than the top angle of the conical surface part of the aperture.

11. An apparatus according to claim 9, wherein the top angle of the conical surface part of the apertures is approximately 10.0° and the top angle of the conical surface part of the containers is approximately 9.7°.

12. An apparatus according to any one of the preceding claims, wherein the holding means is adapted to oscillate or reciprocate the receiving means along an arcuate path.

13. An apparatus according to any one of claims 1-12, wherein the holding means comprises a substantially horizontally

arranged, substantially circular disc which is reciprocatingly movable around its central axis.

14. An apparatus according to claim 13, which comprises a stepping motor drivingly connected to the disc.

15. An apparatus according to claim 14, wherein the disc is mounted directly on the shaft of the stepping motor.

16. An apparatus according to claim 4, wherein the first position of the means for moving at least one container axially is a lower position, and the second position is a higher position.

17. An apparatus according claims 4 or 16, comprising magnet holding means adapted to hold at least one set of magnets in a position where they induce a magnetic field in the sample-receiving part of at least one sample container positioned in its second position.

18. An apparatus according to claim 17, wherein the magnet holding means are adapted to bring, in one position, the magnets in direct contact with the sample-receiving part of the sample container positioned in its second position, and arrange the magnets in another position in which they do not contact the sample container.

19. An apparatus according to claim 17 or 18, wherein the magnet holding means comprise at least two sets of magnets, adapted to be brought in contact with at least two neighbouring sample containers.

20. An apparatus according to any one of claims 17-19, wherein each set of magnets is adapted to contact the sample container from substantially opposite sides.

21. An apparatus according to claim 20 wherein the magnets of each set of magnets contact the sample container with opposite magnetic poles.

22. An apparatus according to any one of claims 17-21, additionally comprising a stationary set of magnets arranged downstream of the magnet holding means.

23. An apparatus according to any one of claims 1-3, which comprises, as means for adding liquid samples to the sample container, pipettes fed by continuous liquid strings extending from containers containing the liquids to the tip of the pipettes.

24. An apparatus according to claim 23, comprising peristaltic pump means for moving liquid from the liquid containers to the pipette tips.

25. An apparatus according to any one of claims 1-3, in which the means for removing liquid from the sample containers comprises suction pipette means combined with flushing liquid supply means adapted to supply flushing liquid to an outer surface part of the suction pipette means.

26. An apparatus according to claim 25, wherein the flushing liquid supply means is adapted to supply flushing liquid to the outer surface part of the suction pipette means through at least one orifice in the suction pipette means positioned at a distance above the tip of the suction pipette.

27. An apparatus according to claim 25 or 26, wherein the flushing liquid supply means comprises an annular cavity surrounding the suction pipette means along a part of the length of the suction pipette means.

28. An apparatus according to any one of claims 1-3 comprising pipette holding and moving means adapted to hold a plurality of pipettes and to move any one of the pipettes to

a position in which the pipette tip is capable of entering a sample container.

29. An apparatus according to claim 28, comprising a number of pipette-holding segments each of which is capable of holding at least one pipette, a segment support means comprising a guide surface on which the segments, arranged adjacent to each other, are supported and guided, means for sidewardly moving the segments, a movable member comprising a support surface adapted to support a segment, the movable member being able to occupy a first position in which its support surface replaces and constitutes part of the guide surface, and means for moving the movable member in downward and upward directions, each segment being sidewardly movable by the segment moving means to a position where the guide surface is replaced with the support surface of the movable member, whereby the segment becomes movable with the movable member, the movable member being movable between the first position and a second position in which the tip of a pipette held by the segment is capable of entering a sample container.

30. An apparatus according to claim 29, wherein the guide surface is in a substantially horizontal plane, and the downward movement is a substantially vertical movement.

31. An apparatus according to claim 29 or 30, wherein the movable member comprises an upper guide surface part adapted to engage an upper surface part of a segment supported by the support surface part of the movable member.

32. An apparatus according to any one of claims 29-31, wherein the segment support means comprises a groove having substantially vertical-parallel edges, the movable member being movable from its first position to its second position along the groove, the segment held by the movable member being fixed in sideward direction by the edges of the groove when the movable member is not in its first position.

33. An apparatus according to any one of claims 29-32, wherein the guide surface of the segment support means constitutes part of a substantially circular surface, the remaining part of the substantially circular surface being constituted by the support surface of the movable member when the movable member is in its first position.

34. An apparatus according to claim 33, comprising a rotatable top part provided with means for engaging the segments supported by the support surface so that the segments are moved sidewards in a substantially circular path when the rotatable top part is rotated, the engaging means being in slidable engagement with the segments allowing a segment which is moved downward with the movable member to become disengaged from the top part.

35. A sample container, in particular for use in the apparatus according to any one of claims 1-34, the sample container having lower and upper substantially cylindrical surface parts connected with intermediate substantially frustro-conical surface part.

36. A sample container according to claim 35, the diameter of the upper substantially cylindrical surface part being larger than the diameter of the lower cylindrical surface part.

37. A sample container according to claim 36, wherein the top angle of the frustro-conical surface part is in the range of 3°-18°, preferably 6°-13°, and more preferably within 9°-11°.

38. A sample container according to any one of claims 35-37, in which the diameter of the lower substantially cylindrical surface part is in the range of 10-15 mm, and the diameter of the upper substantially cylindrical surface part is in the range of 13-19 mm.

39. A sample container according to claim 38, wherein the diameter of the lower substantially cylindrical part is in

the range of 11.5-13.5 mm, and the diameter of the upper substantially cylindrical part is in the range of 15-17 mm.

40. A sample container according to claim 39, wherein the diameter of the lower substantially cylindrical part is about 12.5 mm, and the diameter of the upper substantially cylindrical part is about 16 mm.

41. A sample container according to any one of claims 35-40, in which the lower cylindrical part comprises a downwardly extending substantially hemispherical bottom part.

42. A sample container according to claim 41, wherein the total length of the lower cylindrical part is 25-32 mm.

43. A sample container according to any one of claims 35-42, which is made of polyolefin.

44. A sample container according to claim 43, wherein the polyolefin is polypropylene.

45. A method for agitating one or more liquid samples each contained in a sample container having a portion for containing the liquid sample, said method comprising arranging each of the sample containers in a holding means having container-receiving means in such a manner that each container and each container-receiving means are interengaging in male-female relationship and oscillatingly move the holding means and thereby transferring movement from the holding means to the sample containers.

46. A method according to claim 44, wherein each container is moved axially in relation to the corresponding receiving means between a first position, in which the sample container is received in the holding means with a relatively close fit, and a second position in which the sample receiving part of the container may be moved transversely in relation to the receiving means.

47. A method according to claim 45 or 46, wherein the receiving means are oscillated or reciprocated along an arcuate path.

48. A method according to claim 47, wherein the acceleration of the oscillating or reciprocating movement, in one direction is higher than the acceleration in the opposite direction so as to impart a substantially circular movement to the liquid sample in each container.

49. A method according to any one of claims 45-48, wherein the holding means comprise a substantially horizontally arranged, substantially circular disc which is reciprocatingly moved around its central axis.

50. A method according to claim 48 or 49, wherein the acceleration in one direction of the reciprocating movement is at least two times the acceleration in the opposite direction.

51. A method according to claim 50, wherein the acceleration in one direction of the reciprocating movement is at least 6 times the acceleration in the opposite direction.

52. A method according to claim 50, wherein the acceleration in one direction of the reciprocating movement is at about 9 times the acceleration in the opposite direction.

53. A method according to any one of claims 49-52, wherein the reciprocating movement is obtained my means of a stepping motor which is operated to run at substantially constant speed and is drivingly connected to the disc.

54. A method according to any one of claims 45-53, wherein agitation of the liquid sample in one or some of the containers is substantially prevented or reduced while agitating the liquid samples in the remaining containers.

55. A method according to claim 54, wherein the agitation of the liquid samples in selected containers is substantially reduced or prevented by moving the containers to the second position and then preventing the sample receiving parts of the containers from moving transversely.

56. A method according to claim 55, wherein said first and second positions are lower and upper positions, respectively, one or more of the containers being lifted to their upper position while their lower sample containing parts are being prevented from moving transversely.

57. A method for performing pipetting of liquid into a container by means of a pipette, comprising subjecting the pipette to a movement with an upward acceleration at the end of a pipetting procedure so as to release from the pipette tip any part of any liquid drop in excess of an amount determined by the upward acceleration and allowing any such released amount to be added to the liquid in the container.

58. A method according to claim 57, wherein the movement of the pipette is generated by a motor.

59. A method according to claim 57 or 58, wherein the pipette is fed by a motor-driven pump.

60. A method according to any one of claims 57-59, wherein, at the end of the pipetting procedures, the pipette is first subjected to a downward movement so as to give any liquid drop remaining at the pipette tip a downward velocity, whereupon the pipette is subjected to the upward acceleration.

61. A method according to claim 60, wherein, at the end of a pipetting procedure, the pipette is moved upward to a first position, whereupon the pipette is subjected to the downward movement, which movement is reversed by means of the upward acceleration.

62. A method according to claim 61, wherein, at the end of a pipetting procedure, the pipette is first moved upward to a first position, whereupon the pipette is subjected to a downward movement to a second position and is thereafter again moved upward to the first position, whereupon the pipette is subjected to the downward movement, which movement is reversed by the upward acceleration releasing from the pipette tip any part of any liquid drop in excess of an amount determined by the upward acceleration.

63. A method according to any one of claims 57-62, wherein the pipette is kept free of contact with the liquid in the container.

64. A method for removing a liquid sample from a liquid contained in a container by means of a suction pipette, said method comprising

- immersing the pipette into the liquid,
- sucking the liquid sample into the pipette, and
- flushing the outer surface part of the pipette having been in contact with the container by means of a flushing liquid.

65. A method according to claim 64, wherein the flushing liquid is supplied through at least one orifice in the pipette positioned above the outer surface part of the pipette which has been immersed in the liquid.

66. A method according to claim 64 or 65, wherein the flushing comprises at least one flushing sequence where the flushing liquid is supplied in an amount which is equal to or preferably larger than the amount of liquid sample which has been withdrawn so as to secure that total outer surface part of the pipette which has been immersed in liquid is contacted by the flushing liquid.

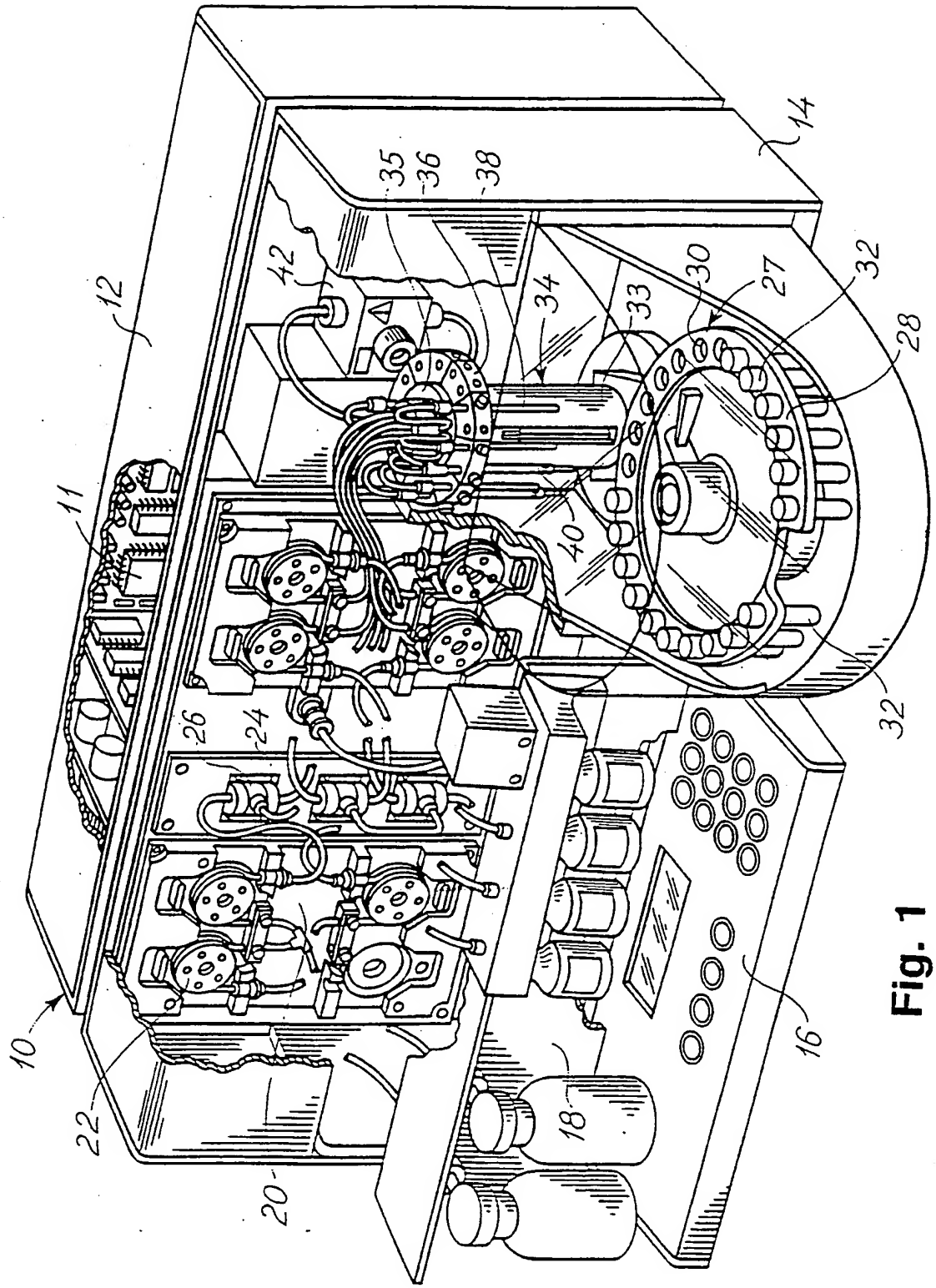

Fig. 1

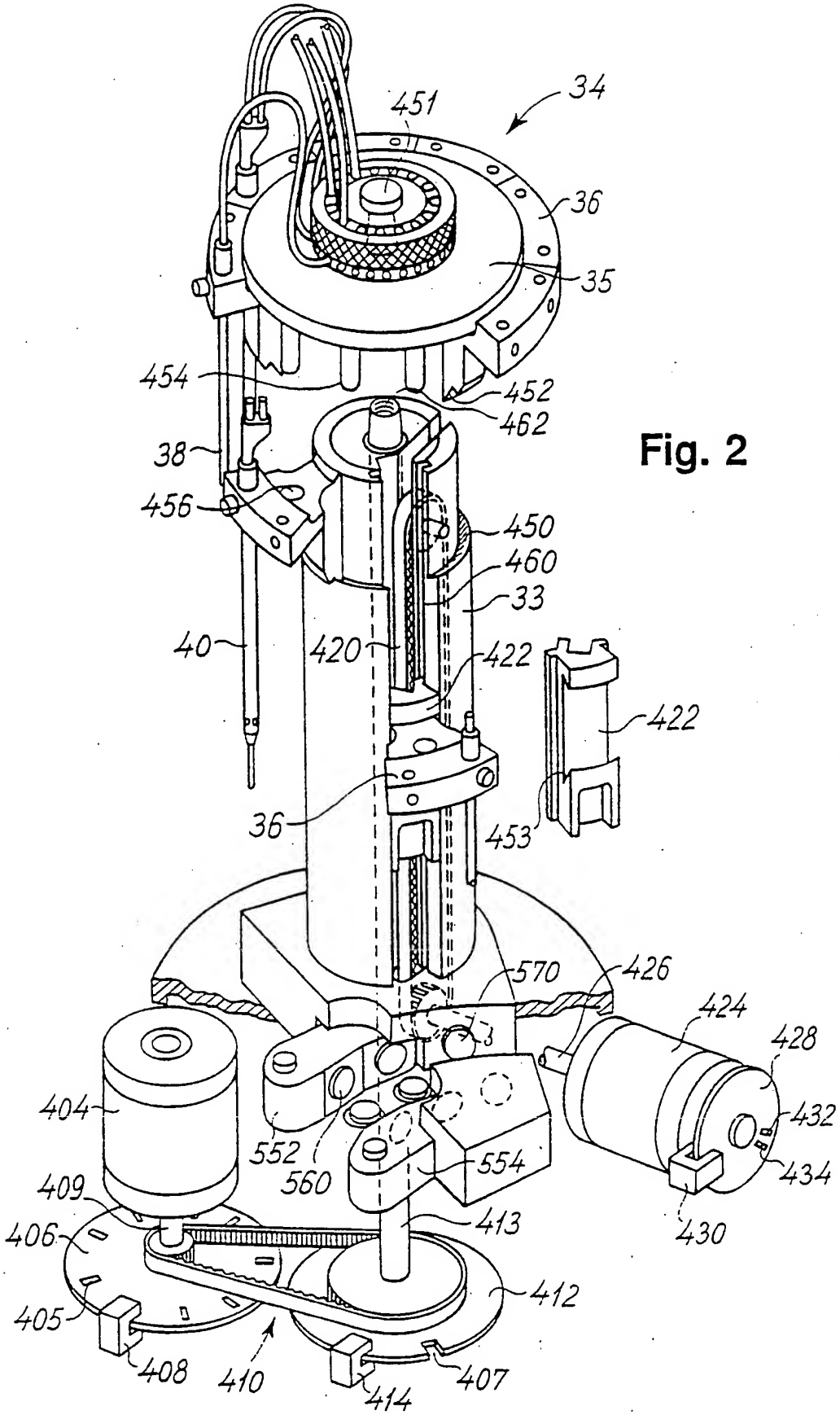
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Fig. 2

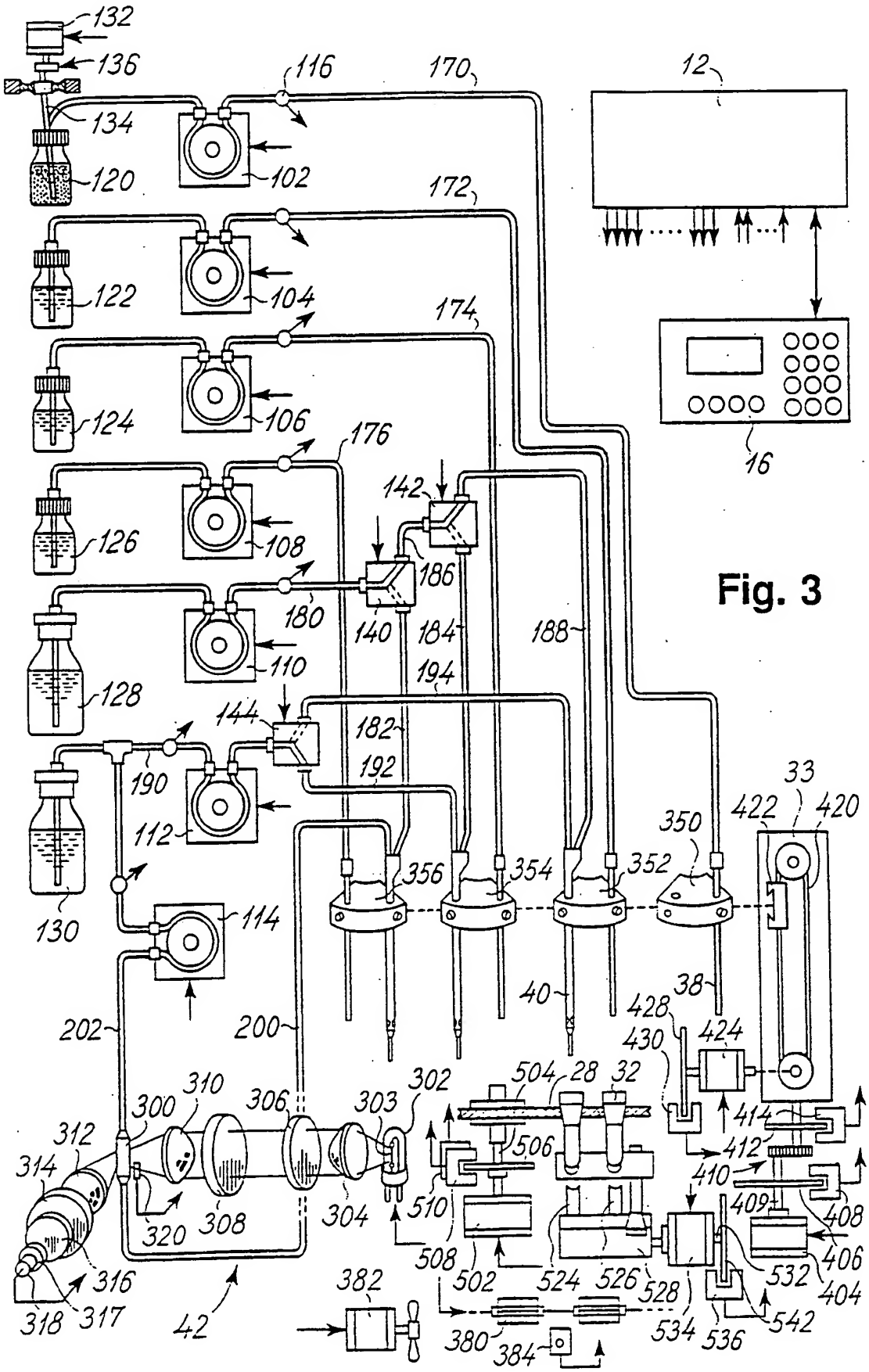
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Fig. 3

Fig. 4

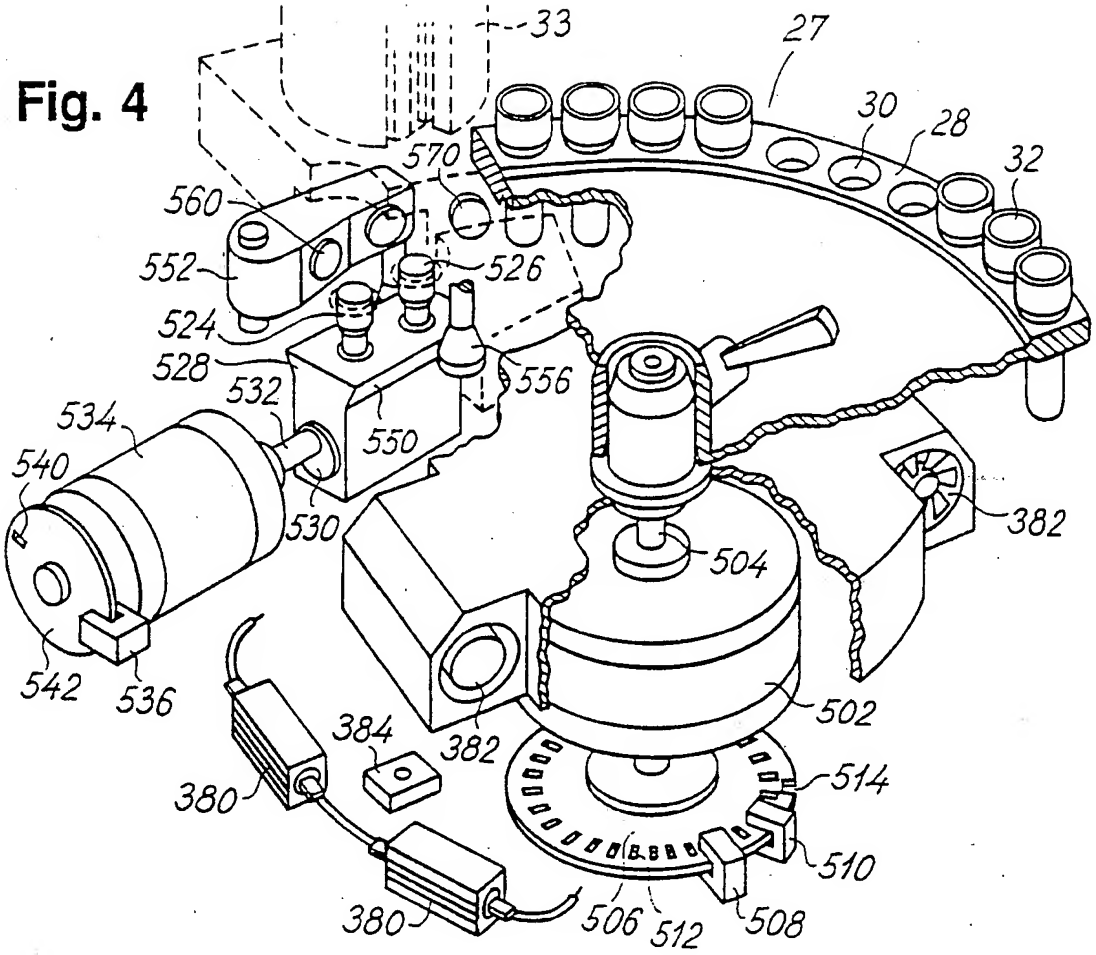


Fig. 5

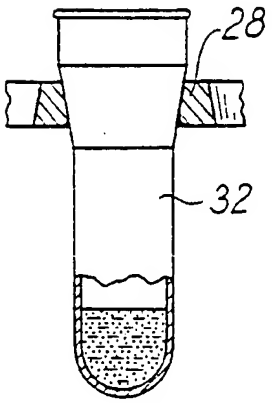


Fig. 6

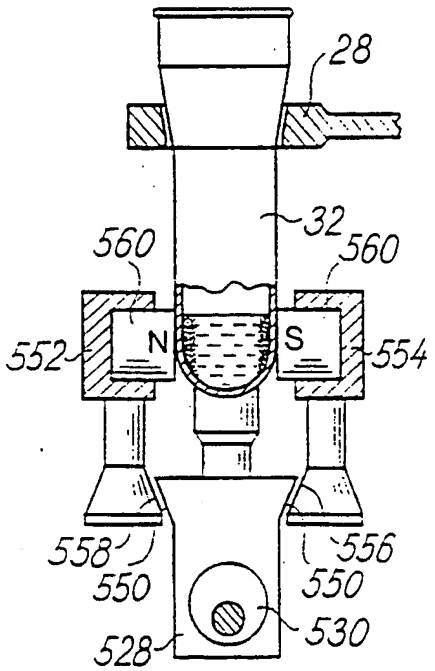
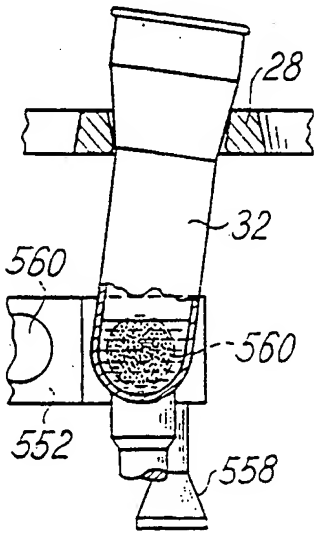


Fig. 7



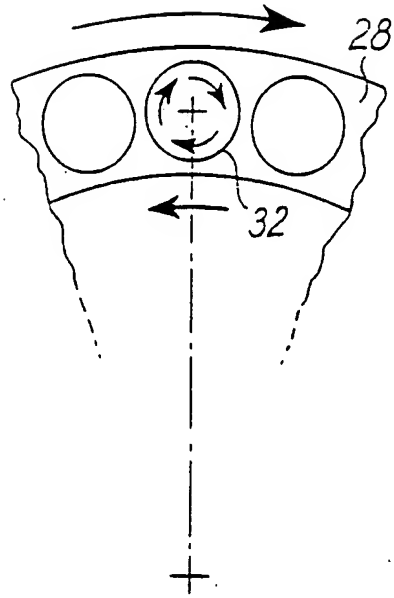


Fig. 8

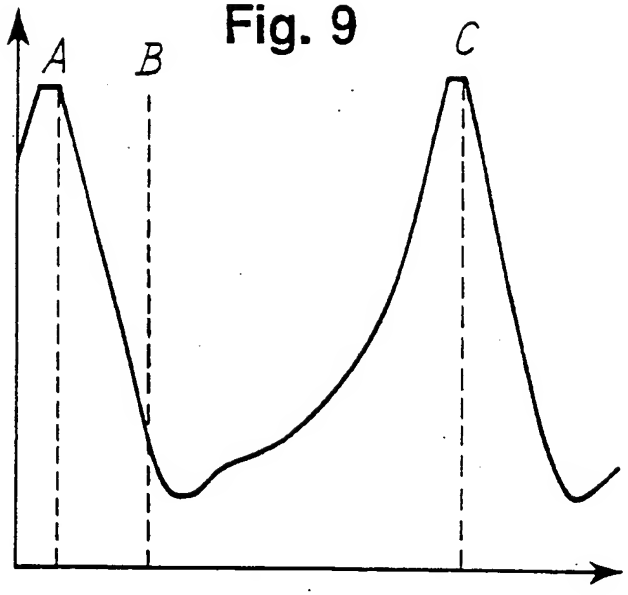


Fig. 9

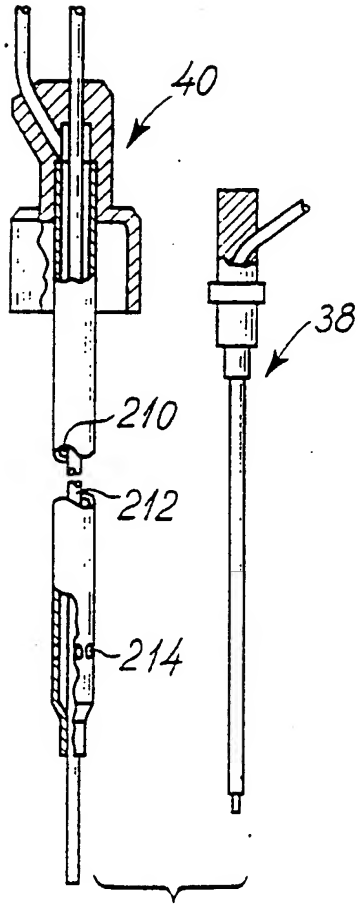


Fig. 10

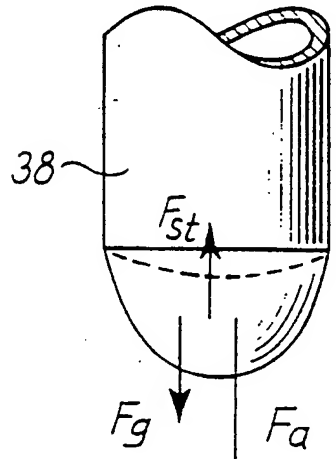


Fig. 11

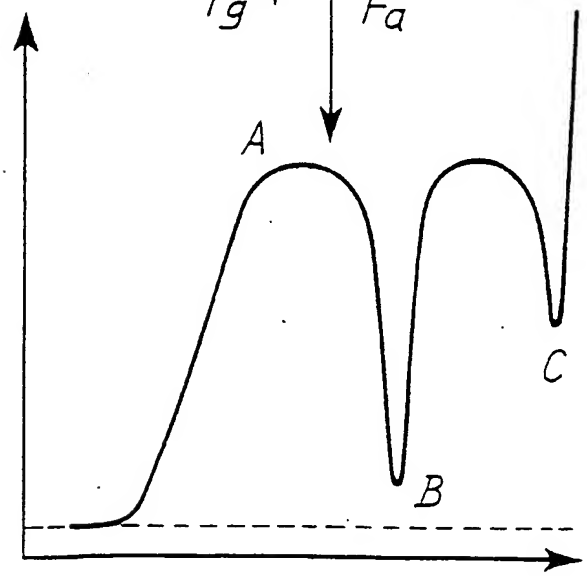


Fig. 12

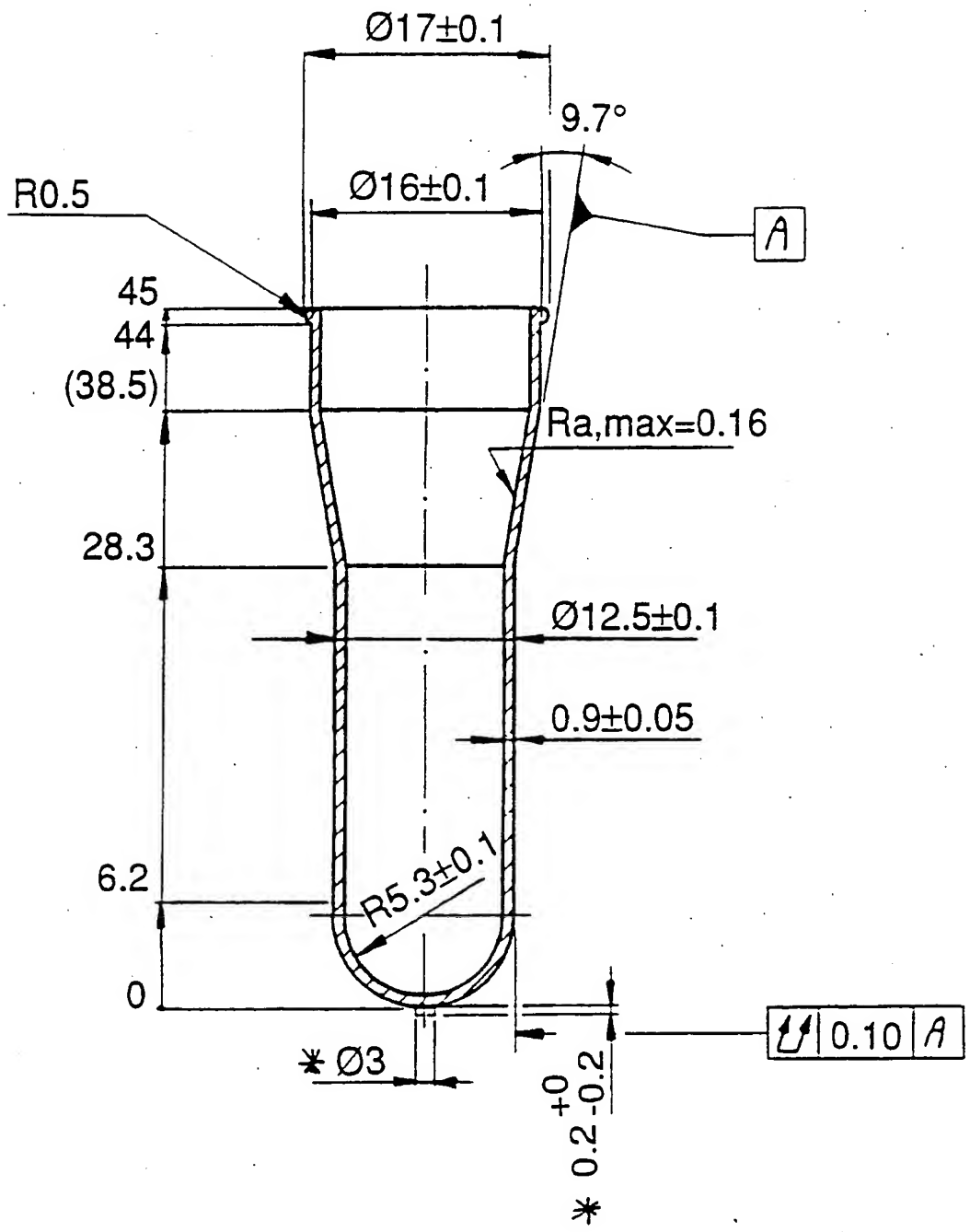

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Fig. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00195

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 35/06, B01F 9/10, B01F 11/00, B01L 3/14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: B01F, B01L, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DE, B2, 2602675 (BODENSEEWERK PERKIN-ELMER & CO GMBH), 10 July 1980 (10.07.80), column 5, line 15 - line 59, figure 1, claim 1 --	1-3,13,23
Y	EP, A1, 0087028 (TOKYO SHIBAURA DENKI KABUSHIKI KAISHA), 31 August 1983 (31.08.83), page 5, line 16 - line 19; page 6, line 35 - page 7, line 30; page 12, line 34 - page 13, line 10, figure 1 --	1-3,13,23
A	DE, B, 2159430 (ROHE SCIENTIFIC CORP.), 31 October 1974 (31.10.74), column 7, line 32 - line 38; column 7, line 65 - column 8, line 2, figure 1 --	1-34

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 1993	08 -11- 1993
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Bengt Christensson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00195

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE, C2, 3015051 (OLYMPUS OPTICAL CO.,LTD.), 28 February 1985 (28.02.85), claim 1	1-34
X	EP, A1, 0146074 (TERUMO MEDICAL CORPORATION), 26 June 1985 (26.06.85), figure 5	35,36,41
X	DE, A1, 3129185 (TECHNICON INSTRUMENTS CORP.), 8 April 1982 (08.04.82), page 12, line 17 - line 29; page 13, line 17 - page 14, line 37; page 19, line 6 - line 24, figures 1-3	45,49,53
Y		1-3,13,23
A	US, A, 2052096 (ALFRED L. KRONQUEST), 25 August 1936 (25.08.36), column 2, line 43 - line 51	1-3,45-56

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00195

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

(See extra sheet 210)

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/DK 93/00195

1/ An apparatus and a method for the handling of liquid samples and agitating liquid samples according to claim 1, part a), corresponding dependent claims 2-34 and claims 45-56.

2/ An apparatus and a method for the handling of liquid samples and performing pipetting operation according to claim 1, part c), corresponding dependent claims 2-34 and claims 57-63.

3/ An apparatus and a method for the handling of liquid samples and removing and flushing liquid according to claim 1, part d), corresponding dependent claims 2-34 and claims 64-66.

4/ A sample container according to claims 35-44.

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/93

International application No.
PCT/DK 93/00195

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE-B2- 2602675	10/07/80	AU-B-	506224	20/12/79
		AU-A-	2153977	27/07/78
		GB-A-	1514443	14/06/78
		US-A-	4068529	17/01/78
EP-A1- 0087028	31/08/83	JP-C-	1664462	19/05/92
		JP-B-	3026348	10/04/91
		JP-A-	58139070	18/08/83
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		SE-A-	8104627	05/02/82
US-A- 2052096	25/08/36	NONE		